



A Novel Fluorescent FRET Hairpin Probe Switch for *aflD* Gene Detection in Real Fermented Soybean Paste

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Received: 3 August 2020 / Accepted: 16 June 2021 / Published online: 6 July 2021
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Abstract

The development of detection method for aflatoxin gene cluster in *Aspergillus flavus* and *Aspergillus parasiticus* is an effective approach to prevent the widespread of aflatoxins, which are considered as extremely, toxic substances. Herein, a hairpin DNA-assisted fluorescence probe switch based on Förster resonant energy transfer (FRET) was designed. In this work, the two terminals of hairpin DNA probe were combined with CdTe/CdS quantum dots as donor and gold nanoparticles (AuNPs) as acceptor respectively to fabricate the FRET hairpin probe switch, which were utilized to detect the aflatoxic fragment of *aflD* genes for the first time. In the absence of target DNA, the hairpin probe formed the double stem loops state to trigger the FRET to induce the fluorescence-quenching (turning off). With the addition of target DNA, the specific interaction between target and hairpin probe switch brought about the separation of fluorescent energy donor–acceptor pairs to create an increasing fluorescence response (turning on). Consequently, the fluorescence intensity recovers can be employed to detect *aflD* genes in homogenous phase through optical responses. This proposed FRET hairpin probe switch performed a great limit of detection of 0.02 nM with a linear range of 0.05–200 nM under optimal conditions. Furthermore, the feasibility of the fabricated strategy was demonstrated in fermented soybean paste with the advantages of simplicity, rapidity, and sensitivity, which indicated that this method exhibited the potential and practicability in foodborne biological samples.

Keywords FRET switch · Hairpin probe · Quantum dots · AuNPs · *aflD* genes

Introduction

Mycotoxins are highly toxic metabolites, which frequently contaminate food to provoke the major concerning of food safety in the world (Dalie et al. 2010; Kollia et al. 2017; Sardinias et al. 2011). Especially the aflatoxins, which are kinds of mycotoxins, are considered as the most violent in toxicity due to that they can cause potent chronic toxicity in humans and recognized as the carcinogens causing mortality

(Donner et al. 2010; Khaleghipour et al. 2020). Therefore, various determination methods were fabricated to detect the aflatoxins in food including chromatography, (Madurangika Jayasinghe et al. 2020; Thomas et al. 2016), enzyme-linked immunosorbent assay (ELISA) (Peng et al. 2020; Shephard 2008), and biosensor (Geleta et al. 2018; Guo et al. 2018). Additionally, biosensors based on signal-labeled can effectively improve the detection sensitivity and apply for target gene identification (Li et al. 2020a; Wu et al. 2021). Aflatoxins were mutagenic secondary metabolites produced by the *Aspergillus* genus and the potent hepatotoxin of aflatoxins is associated with the *aflD* genes of aflatoxin gene cluster from *Aspergillus flavus* and *Aspergillus parasiticus* which both are the main aflatoxin producers (Scherm et al. 2005). Therefore, the existence of *aflD* structural genes can be employed, as the marker of aflatoxin producers and the development of a simple, rapid, and sensitive method for aflatoxic species detection, which can play an important role to ensure the safety of food (Abdel-Hadi et al. 2010; Sedighi-Khavidak et al. 2017).

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The most common method for identifying *afID* genes is polymerase chain reaction (PCR) method, including real-time quantitative PCR and multiplex PCR which generally require sample enrichments, expensive reagents, and trained personnel (Cruz and Buttner 2008; Yunes et al. 2020). It is necessary to develop the effective, simple, and sensitive analysis strategy to improve aflatoxigenic identification, control the greater widespread pollution of aflatoxin, and reduce the possibility of threatening human health.

Förster resonant energy transfer (FRET) was nominated on its discoverer's name Theodor Förster, who was a German scientist (Förster. 1948). There are three key factors to fabricate a FRET system, including energy donor and acceptor FRET pairs, micro-distances, and degree of spectral overlap. FRET was triggered once the energy was transferred from one excited energy donor to another acceptor molecule, via intermolecular dipole–dipole interaction in close proximity (Xu et al. 2014; Zamborini et al. 2012). The FRET biosensor employing the quantum dots (QDs) as the donor has attracted great interest due to its excellent features such as broad excitation spectral, merit simplicity, tunable spectral, and high fluorescent yields (Algar et al. 2011; Dezhurov et al. 2011; Li et al. 2019; Mo et al. 2020). Meanwhile, various QDs including but not limited to magnetic QDs (Wang et al. 2019), ZnS QDs (Liu et al. 2019), CdSe QDs (Hao et al. 2019), carbon QDs (Jalili et al. 2020), and CdTe QDs (Yang et al. 2021; Zhai et al. 2016) were investigated gradually to fabricate kinds of biosensor for poisonous substance detection in food safety field. In order to obtain high sensitivity, it is critical to improve the fluorescent stability and quantum yield which are strongly dependent on the properties of their surface. As a result, the poor surface passivation and surface defects can lead to enhanced trap state emission or non-radiative recombination and generate a low fluorescence quantum yield and photo-bleaching (Lee and Osborne 2009; Medintz et al. 2005). To overcome these issues, QDs were designed and applied to allow large improvements in photoluminescence and increased thermal and chemical stability (Deng and Guyot-Sionnest 2016; Guo et al. 2003). The benefit of using heterostructured QDs as a FRET donor in biosensors has the potential to extent significantly the candidate pool to enlarge the overlapping in donor–acceptor pair, which could efficiently enhance the effect of energy transfer and obtain favorable stability (Cirillo et al. 2014).

Inspired by the merit fluorescent property of CdTe and the advantageous stability of the heterostructured nanomaterials in our previous studies (Li et al. 2020b; Li et al. 2017), the homogeneous FRET hairpin probe switch based on CdTe/CdS–AuNPs donor–acceptor pair and hairpin DNA was developed to detect *afID* genes, which is an aflatoxin gene clustered in *Aspergillus flavus* and *Aspergillus parasiticus*, for the first time. The novel fluorescent biosensor was constructed successfully on account of DNA-specific

recognition between target DNA and hairpin probe. It was credited to the hairpin DNA, which was an optimal candidate for making the opportunity to bring donor–acceptor pair together (Farjami et al. 2011; Jockusch et al. 2006). In this developed strategy, an obvious FRET occurred to induce the fluorescent signal “turn off.” With the addition of a series of target *afID* gene, the fluorescent signal was “turned on” in varying degrees accordingly to achieve the goal of quantitative determination *afID* gene under optimal conditions. This proposed homogeneous FRET hairpin probe switch performed excellent selectivity, repeatability, and reliability. Furthermore, this fabricated FRET hairpin probe switch was utilized in *afID* gene PCR product detection and achieved a good result, which indicated this method had the potential capability to be the feasible tool for the *afID* gene determination in the real fermented soybean paste samples.

Experimental

Agents and Apparatus

The main chemical agents for CdTe/CdS QDs synthesis were as follows: Tellurium powder (99%), CdCl₂·2.5H₂O (99%), NaBH₄ (99%), 3-mercaptopropionic acid (MPA, 99%), thioglycolic acid (TGA, 98%), thioacetamide (TAA, 98%), and sodium hydroxide (NaOH, 96%). The main chemical agents for AuNP synthesis were as follows: HAuCl₄·4H₂O (47.8%) and sodium citrate solution (98%). All of the above-mentioned agents were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). The main chemical agents for construction fluorescent probe were as follows: Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and N-hydroxysuccinimide (NHS). They were purchased from Sigma-Aldrich (Shanghai, China). All reagents in this study belonged to analytical grade and were used without any further purification. All DNA sequences were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). The sequences of DNA used in this work are listed in Table 1. The distilled water was used in the whole experiments. The main apparatus for this work were as follows:

Table 1 The oligonucleotides' sequences of this work sequences

Name	Oligonucleotides sequences (from 5' to 3')
Hairpin DNA probe	H ₂ N-CGGCCG-CTACCAGGGGAGTTG AGATCC-CGGCCG-(CH ₂) ₃ -SH
Target <i>afID</i> gene	GGATCTCAACTCCCCTGGTAG
2 mismatched DNA	GGATGTCAACTCCGCTGGTAG
5 mismatched DNA	GTATCTGAACTGCCATGGTAG
Random DNA	CTGCATGTAGTAATGCCACGT

JEOL 2100 TEM (JEOL, Japan), UV-2450 spectrophotometer (Shimadzu, Japan), Hitachi F-4500 fluorescence spectrophotometer (Tokyo, Japan).

Synthesis of CdTe/CdS QDs

The synthesis of CdTe/CdS QDs was prepared according to the method reported before with brief modification (Li et al. 2017; Zhu et al. 2018). The synthesis procedure of CdTe/CdS was as follows: First, 0.0449 g NaBH₄ and 0.0638 g tellurium powder were added to 5 mL of water, under nitrogenous atmosphere and stored in fridge for 12 h. In this step, tellurium powder was reduced by the NaBH₄ and the fresh NaHTe was generated continuously. Meanwhile, 100 μL MPA and 0.1142 g CdCl₂·2.5H₂O were dissolved in 50 mL water in three necked bottles and 1.0 M NaOH was used to adjust the pH value of the solution to pH 9.0. Secondly, 2.5 mL NaHTe solution was injected rapidly into the above mixture solution under 2000 rpm vigorous stirring and protected by nitrogen atmosphere. And the mixture solution was heated at 100 °C for 30 min to gain light yellow CdTe QDs. And then, the CdTe QDs were precipitated with excess absolute ethyl alcohol (volume ratio = 1:4) at 10,000 rpm for 5 min to remove unreacted substance. The lower CdTe QDs sediments were redispersed in the same volume of water and stored less than 4 °C in fridge for subsequent use. Thirdly, 190 mg CdCl₂·2.5H₂O and 64.15 mg TAA were used for cadmium and sulfur source respectively and resolved simultaneously in 100 mL water to fabricate S and Cd stock solution. Followed by adding 100 μL 10 M TGA into the latter solution to be stabilizer. The pH value of the solution was adjusted to pH 12.0 by 1.0 M NaOH. Finally, 5 mL as-prepared pure CdTe QDs was added into 50 mL water which was heated to 100°C. Subsequently, 10 mL prepared S and Cd stock solution were added into the above hot solution and continued to be stirred to obtain epitaxial growth of CdS QDs. The mixture solution was refluxed 40 min at 100 °C to form CdTe/CdS QDs. The purification procedure was similar to CdTe QDs and the purified CdTe/CdS QDs were redispersed in the same volume of water and stored under 4 °C in fridge for further use.

Preparation of CdTe/CdS-HP Probe

Two milliliters as-prepared CdTe/CdS QDs was dispersed in 5.0 mL 25 mM PBS buffer (pH 7.4). Followed by adding 38.0 mg NHS and 76.0 mg EDC to activate the surface carboxylic group for 40 min under even stirs at 25 °C temperature. Subsequently, 10.0 μL 100.0 μM hairpin DNA was added into the above mixture solution and continued to whisk gentler for further 24 h. The resulting mixture was centrifuged at 10,000 rpm for 8 min and washed 3 times with 25 mM PBS buffer (pH 7.4) to wipe excessive NHS

and EDC to obtain CdTe/CdS-HP probe. The eventual precipitate of CdTe/CdS-HP probes was dispersed in the same volume water and stored at 4 °C for later use.

Synthesis of AuNPs

AuNPs were prepared according to the previous reported reduction method (Ye et al. 2014). Briefly, a mixture of 2 mL HAuCl₄ (2.0 wt%) and water (98 mL) was added and boiled at 2500 rpm vigorous stirring in a clean beaker which was washed thoroughly with aqua regia (the volume ratio of HNO₃:HCl at 1:3 ratio) and rinsed with water. Subsequently, 1 mL sodium citrate solution (2.0 wt%) was added into the boiled mixture solution within 2 s under the same stirring rate. The color of the solution changed from light fuchsia to deep wine red in a few minutes. The solution was percolated by the 0.8-μm membrane filter after it was boiled for around 15 min and cooled it to room temperature. Finally, the prepared AuNPs were dispersed in 50 mL water and stored at 4 °C for the later use.

Construction of Homogeneous FRET Hairpin Probe Switch

The homogeneous FRET hairpin probe switch was constructed by linking the prepared thiol-modified CdTe/CdS-HP probe and AuNPs. The detailed operation process was following the pre-literature described elsewhere (Wang et al. 2017). The thiol-modified CdTe/CdS-HP probe was incubated with 2.0 μL TCEP (15 mM) for 1 h to cleave disulfide linkage under gentle vibration. Then 100 μL activated CdTe/CdS-HP probe and 900 μL AuNPs were mixed and incubated for 24 h at 25 °C temperature with gentle stirring. The final solution was centrifuged at 10,000 rpm for 8 min 3 times with deionized water and the homogeneous FRET hairpin probe switch was obtained by re-dispersing the precipitation in the same volume of water.

The Detection of *afID* and Application in Real Fermented Soybean Paste

The fabricated homogeneous FRET hairpin probe switch was employed to detect *afID* by the specific recognition between hairpin probe and target *afID* gene. Briefly, a series of 200 μL target *afID* genes with different concentrations were added to 1.0 mL prepared FRET hairpin probe switch solution to incubate for 40 min to trigger the fluorescence signal “turn on.” The final solutions were diluted to 2.0 mL respectively and measured the fluorescence intensity by fluorescent spectrophotometer under 360-nm excitation wavelength. The various mismatched gene sequences were used to assess the selectivity of the FRET hairpin probe switch. Additionally, the real DNA samples were extracted from

fermented soybean paste by the Plant Genomic DNA Kit, (Sangon Biotech Co., Ltd. Shanghai, China) and amplified by PCR method. Subsequently, 200- μ L PCR amplification products were employed to estimate the utility of the fabricated homogeneous FRET hairpin probe switch.

Results and Discussion

Mechanism of FRET Hairpin Probe Switch for *aflD* Detection

Donor and acceptor coexisting in the homogeneous solution with in adjacent distance could trigger the FRET. In this study, the hairpin DNA whose two end sequence was conjugated with the amino and sulfhydryl group respectively was chosen to be the bridge for FRET. Meanwhile, the AuNPs were used to be an acceptor and CdTe/CdS QDs were employed as a donor contributed to its superior and stabilized fluorescent characters. The heterostructured CdTe/CdS QDs were immobilized with the amino terminus of hairpin DNA by the reaction of amides to form CdTe/CdS-HP and gain the strong fluorescent signals to turn fluorescent signal “turn on.” Simultaneously, the AuNPs was linked with other terminal of CdTe/CdS-HP by the strong affinity of Au–S bonding, which approximated the distance between CdTe/CdS and AuNPs; it provided the chance for FRET occurrence from the CdTe/CdS to AuNPs, leading to the FRET

hairpin probe switch showing the fluorescent signal “turn off” state. The principle of the FRET hairpin probe switch is shown in Scheme 1. The presences of the target *aflD* gene could hybrid with hairpin probe and extended the distance between donor and acceptor as far as possible, which could reduce energy transfer to form the “open” beacon state and turned fluorescent signal “turn on.”

Characterization of CdTe/CdSQDs and AuNPs

To estimate the properties of CdTe/CdS QDs and AuNPs, the TEM images of CdTe/CdS and AuNPs were taken to study the morphology and size. It was shown in Fig. 1A, AuNPs were in a great-dispersed state with the approximate round and 13-nm average diameter, which was beneficial for FRET occurrence. TEM image of CdTe/CdS QDs and high-resolution (HR) TEM image of CdTe/CdS QDs are shown in Fig. 1B and Fig. 1B (a), the crystal structure of CdTe/CdS QDs was clearly visible, and the formation of the CdS QDs on the CdTe surface is consistent with previous reports (Yue et al.2016; Jing et al.2015). The high-angle annular dark-field scanning TEM (HAADF-STEM) image and EDX were obtained and as shown in Fig. 1C and D. The elemental mapping (Fig. 1C (a–d) and profile analyses by energy-dispersive spectroscopy (EDS) of CdTe/CdS indicated containing Cd, Te, and S, which illustrated the successful preparation of CdTe/CdS quantum dots. The spectral overlapping between CdTe/CdS QDs and AuNPs was researched by normalized

Scheme 1 Schematic illustration based on FRET between CdTe/CdS QDs and AuNPs to detect the *aflD* gene of production aflatoxin

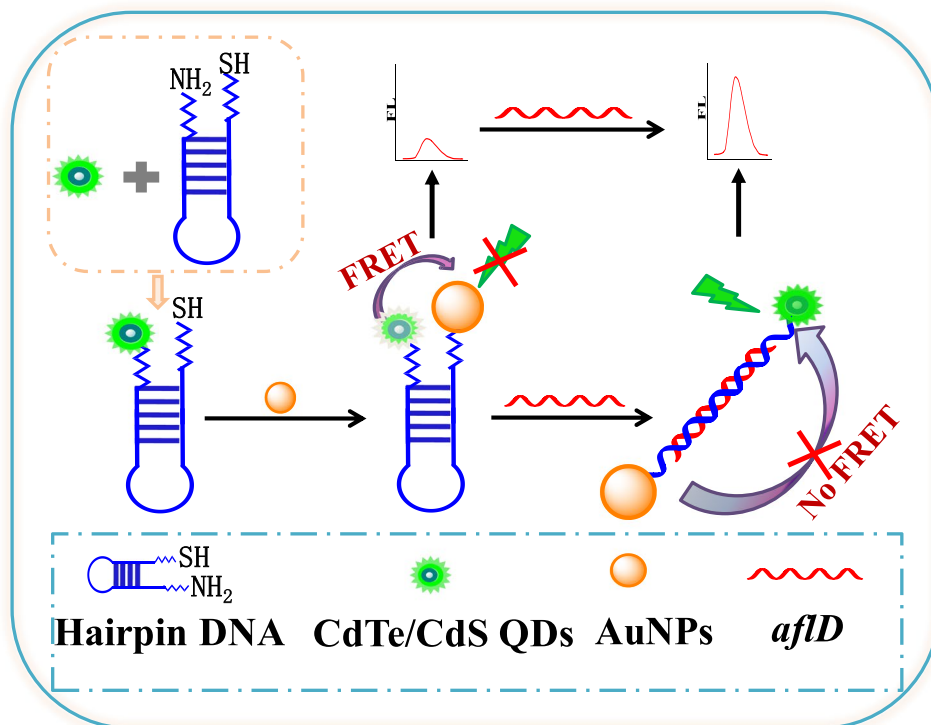
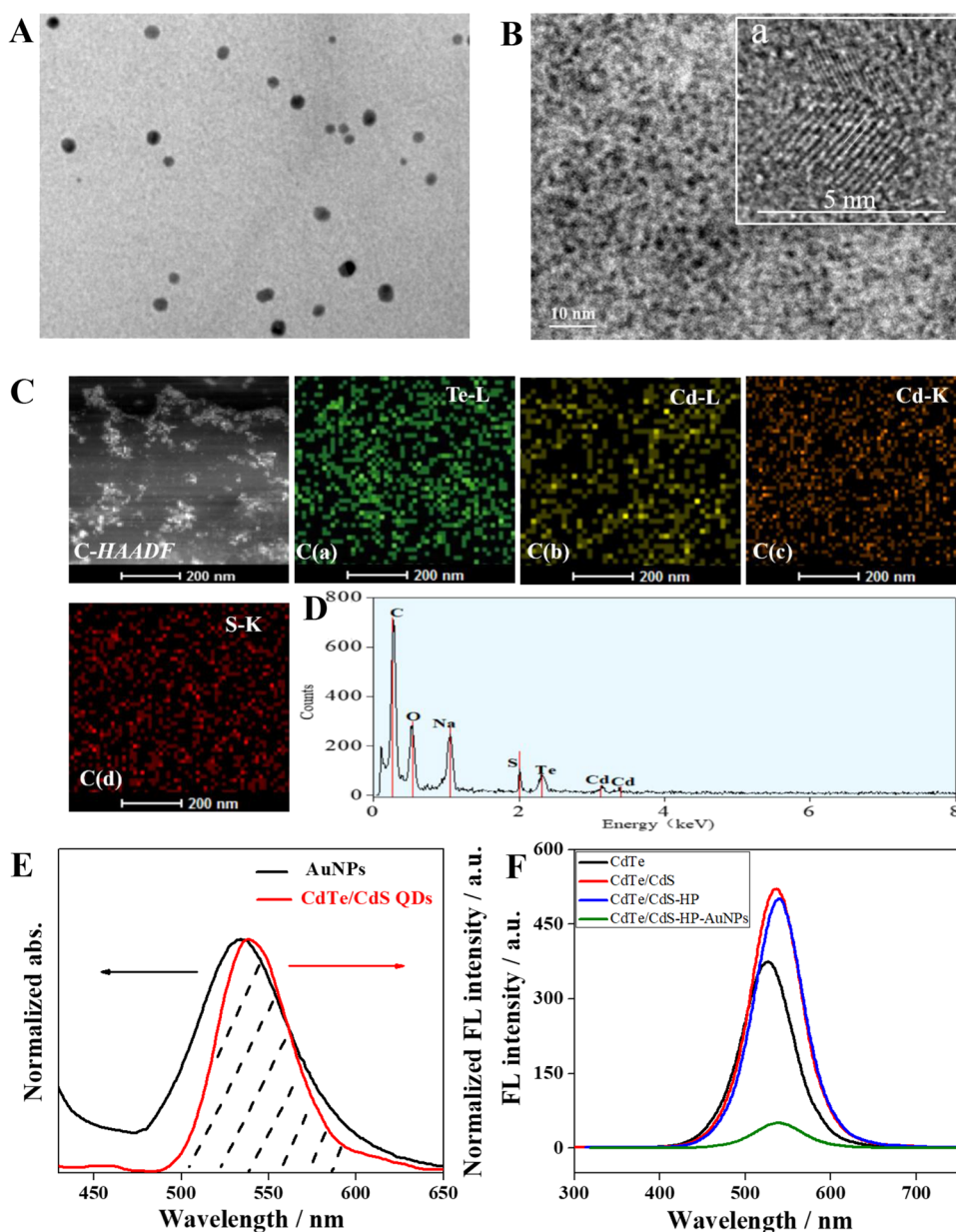


Fig. 1 Characterization of AuNPs and CdTe/CdS QDs, the TEM image of AuNPs (A); TEM image of CdTe/CdS QDs (B) and HR-TEM image of CdTe/CdS QDs (B (a)); HAADF-STEM image and corresponding elemental mapping analysis of CdTe/CdS from top view (C-HAADF). Green, yellow, orange, and red colors indicated Te-L (C(a)), Cd-L (C(b)), Cd-K (C(c)), and S-K (C(d)) respectively; EDS of CdTe/CdS (D); Spectra overlapping between normalized emission spectra of CdTe/CdS and normalized absorption spectra of AuNPs (E); Fluorescence spectra of CdTe, CdTe/CdS, CdTe/CdS-HP, and CdTe/CdS-HP-AuNPs (F)



analysis method and it was performed in Fig. 1E. The result illustrated the ultraviolet absorption wavelength of AuNPs was 530 nm and the fluorescent emission wavelength was 539 nm under the exciting wavelength of 360 nm. The diameter of synthesized CdTe/CdS QDs can be calculated as previous report and followed (Zhu et al. 2018):

$$D = (9.8127 \times 10^{-7})\lambda^3 - (1.7147 \times 10^{-3})\lambda^2 + (1.0064)\lambda - 194.84$$

In this equation, D (nm) is the diameter size of the given nanoparticles, and the λ is the first excitonic peak of the CdTe/CdS QDs. According to the above equation, the corresponding diameter size of CdTe/CdS QDs was around 3.1 nm. Moreover, the overlapping of the two spectral

obviously exhibited overwhelming extent, which provided the advantageous condition for the homogeneous FRET hairpin probe switch.

Spectral Properties of CdTe/CdS, CdTe/CdS-HP, and FRET Hairpin Probe Switch

The fluorescent properties of CdTe/CdS, CdTe/CdS-HP, and FRET hairpin probe switch were the critical indicators for this proposed strategy; therefore, it was necessary to study the fluorescent properties of various steps. As shown in Fig. 1F, the CdTe performed certain fluorescent intensity; meanwhile, the fluorescent intensity increased after the CdTe was capped by CdS to form CdTe/CdS QDs, which

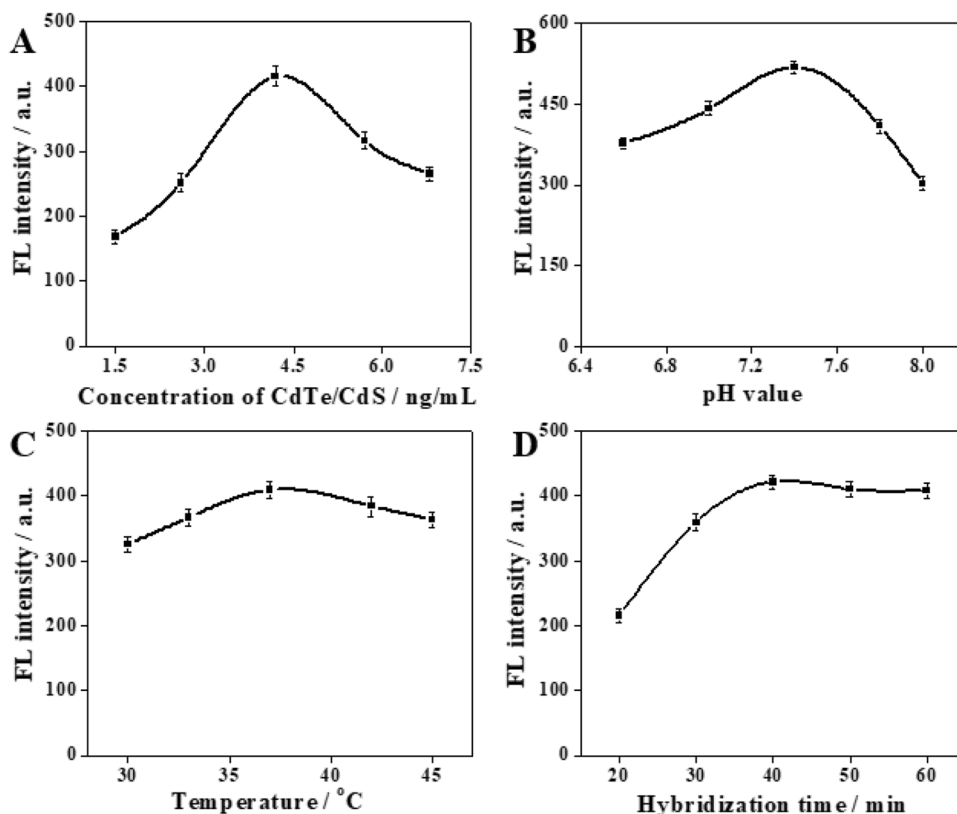
was contributed to the heterostructured structure enhancing stability of QDs. While the hairpin DNA probe was combined with CdTe/CdS QDs to form CdTe/CdS-HP, there was no obvious change of the fluorescent intensity; it could be observed a slight red shift in emission spectra, which demonstrated the successful conjugation. Additionally, the fluorescent intensity had greatly reduced when the AuNPs were linked with CdTe/CdS-HP, and it attributed to the triumphant constitution of FRET hairpin probe switch and the energy transfer from the donor of CdTe/CdS QDs to the acceptor of AuNPs. The successful FRET hairpin probe switch placed the foundation and opportunity for the target DNA detection.

Optimization of Assay Conditions for *afID* Detection

To achieve the excellent test condition, the important experimental parameters of concentration of CdTe/CdS QDs, pH values, reaction temperature, and hybridization time of DNA were optimized respectively. First of all, the CdTe/CdS QDs was employed as the fluorescent signal as it was significant to optimize its concentration. Therefore, the concentration of CdTe/CdS QDs was studied by in the range of 1.5–6.8 ng/mL and shown in Fig. 2A. The result illustrated that the fluorescent intensity increased with the CdTe/CdS QD addition in the initial stage and began to decrease from the 4.2 ng/mL; therefore, the 4.2 ng/mL was chosen to be the best

concentration of QD donor. Secondly, the pH value of the detection system was closely related to the stability of the FRET hairpin probe switch, which was necessary to study pH environment. The proposed method was carried out in different pH values such as 6.6, 7, 7.4, 7.8, and 8, and the experimental result is shown in Fig. 2B; an increasing tendency of fluorescent intensity was observed until pH 7.4 and the curve went down. Hence, the pH value of 7.4 was used as the buffer with the optimum pH for the later detection. Furthermore, the reaction temperature and hybridization time were the most important elements to reduce the detection speed, hence, then need to investigate them. Consequently, the effect of reaction temperature was also investigated in various temperatures between 33 and 45 °C. As illustrated in Fig. 2C, the fluorescence intensity increased with the extension of reaction temperature and achieved a maximum level at 37 °C. In consequence, the 37 °C was employed to be the optimal reaction temperature. Finally, the hybridization time of the target DNA and FRET hairpin probe switch had a direct bearing on the fluorescence signal turning “on.” To improve the detection efficiency, the hybridization time of 20 min, 30 min, 40 min, 50 min, and 60 min was estimated respectively and presented in Fig. 2D. The intensity of fluorescence signal turning “on” advanced gradually with the extension of hybridization time and there was no obvious change from 40 min. Hence, 40 min was selected as the high efficiency in subsequent experiments.

Fig. 2 Parameter optimization for the *afID* gene of production aflatoxin FRET detection platform. **(A)** Evaluation of the effect of CdTe/CdS concentration; **(B)** Evaluation of the effect of pH value; **(C)** Influence of temperature to the proposed *afID* gene detection strategy; **(D)** Evaluation of the hybridization time



Construction of Homogeneous FRET Hairpin Probe Switch for *aflD* Detection

The proposed homogeneous FRET hairpin probe switch was employed for *aflD* detection under the optimal conditions by adding different concentrations of 0 to 200 nM. When the target *aflD* gene was added into this detection system, the FRET hairpin probe switch was turned to signal “on” state. As illustrated in Fig. 3A, fluorescence intensity was increasing gradually with the addition of target *aflD* gene. Meanwhile, the linear regression analysis of response value from various concentrations was performed to verify the reliability and feasibility of this FRET hairpin probe switch. It was presented in Fig. 3B, the linear range for target *aflD* gene was obtained in the concentration range of 0.05–200 nM and established the standard regression equations: $y = 105.0789x + 195.45$ with the correlation coefficient $R^2 = 0.9948$. The low limit of detection (LOD) for *aflD* gene was 0.02 nM. This result demonstrated this strategy exhibited the excellent linear relation.

This work was compared with previous reported results of other recent literatures (Sedighi-Khavidak et al. 2017; Tombelli et al. 2009), and the result of comparison illustrated our work displayed a lower LODs and broader linear ranges, which is good for the practical application of this homogeneous FRET hairpin probe switch.

Specificity and Repeatability of the Proposed FRET Hairpin Probe Switch

In order to verify the specificity of this FRET hairpin probe switch, mismatched DNA sequence was added into the fabricated FRET hairpin probe switch solution to measure the fluorescent intensity. As shown in Fig. 4, the detection system of perfectly complementary DNA for the hairpin probe exhibited maximum fluorescence recovery, which attributed to DNA base pairing to enlarge the further distance between CdTe/CdS and AuNPs. With the number of mismatched bases increasing, the degree of fluorescence

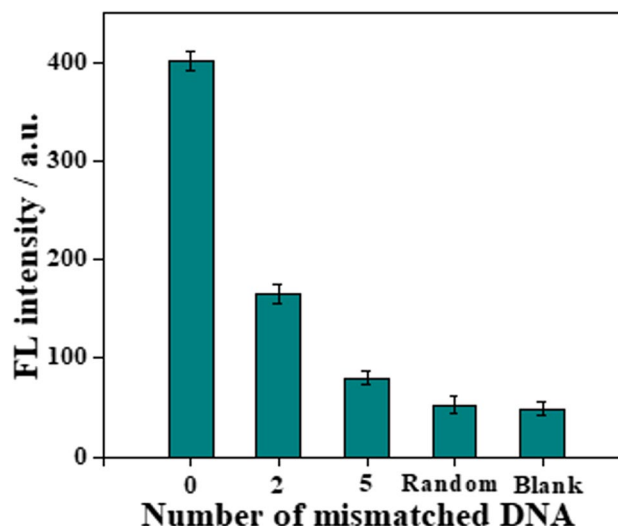


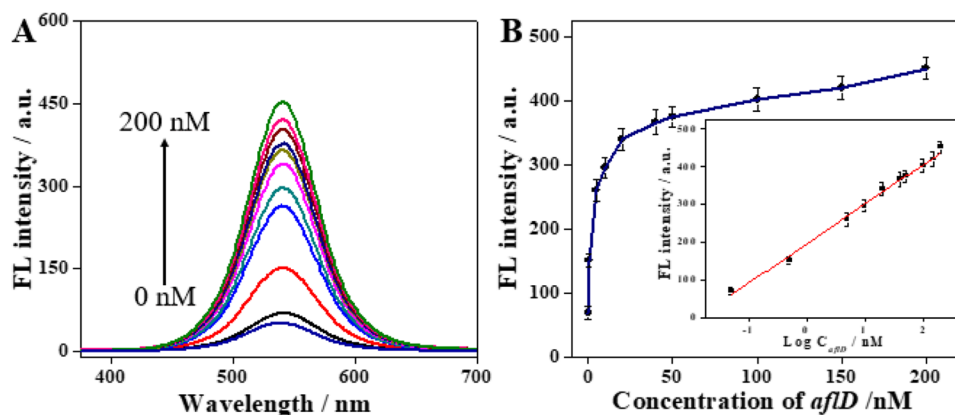
Fig. 4 Analysis of the fluorescence intensity with the increased number of mutations on the target

recovery accordingly reduced as expected. What’s more, the fluorescence recover from response of random DNA sequence was not observed clearly and the fluorescent intensity was approximate with blank group. It was demonstrated this proposed method performed the excellent specificity. To evaluate the repeatability, this proposed FRET hairpin probe switch was employed for 100 nM target *aflD* gene detection in five independent experiments, and the relative standard deviation (RSD) was obtained at 4.36%, illustrating the proposed method possessing great reproducibility. This was good for the utilization in complex sample with the advantaged selectivity and repeatability.

Feasibility of the Develop Strategy in Fermented Soybean Paste

To demonstrate to the feasibility of the fabricated strategy in actual detection, this method was applied to determine *aflD* gene fermented soybean paste samples. The extracted

Fig. 3 Sensitivity results and data analysis. (A) Fluorescence spectroscopy experiments on the different concentrations of *aflD* gene; (B) Linear regression analyses of *aflD* gene detection



DNA sample by the Plant Genomic DNA Kit was amplified with the traditional PCR, and this developed FRET hairpin probe switch displayed effective correspondence for the PCR product in the range of 0.087–6.25 ng/ μ L. The accuracy of *afID* gene detection in fermented soybean paste samples was also evaluated by determining the recovery of *afID* gene by a standard addition method and the recovery values were calculated as 89.6%. Therefore, the results validated this developed FRET hairpin probe switch revealed the outstanding reliability in real fermented soybean paste samples.

Conclusion

In conclusion, a new strategy based on heterostructured CdTe/CdS and AuNP donor–acceptor pairs was developed for *afID* gene detection and applied in the real fermented soybean paste samples. In this strategy, the FRET hairpin probe switch was fabricated through donor–acceptor pairs combined with hairpin DNA probe. Meanwhile, the satisfactory spectra overlapping of donor–acceptor pairs provided guarantee for formation of the high efficiency FRET to measure the target *afID* gene. When the *afID* gene was presented, the remarkable fluorescent recovery was performed with the wide linearity range of 0.05–200 nM and LOD of 0.02 nM. Moreover, various numbers of mismatched base DNA sequence were selected to demonstrate the specificity and five independent experiments were used to evaluate the repeatability; the results displayed the good selectivity and reproducibility. Additionally, the reliability and the feasibility of this developed strategy were demonstrated in PCR product of fermented soybean paste samples. Therefore, all this mentioned above accounted for this method had the potency to apply in the gene segment detection from fungal strains and pathogenic bacterium.

Funding This work was financially supported by the National Natural Science Foundation of China (No. 31901799), the Natural Science Foundation of the Jiangsu Higher Education Institutions of China (Nos. 19KJB550006, 18KJB550003), the Natural Science Foundation of Jiangsu Province (No. BK20180979), the Research Foundation of Zhenjiang Science and Technology Bureau (No. FZ2017068), the Central Public-interest Scientific Institution Basal Research Fund (No. 1610212018005), and the Research start-up fund of Jiangsu University of science and technology (Nos. 1182931701, 1182931801, 1182932001, 1182931804).

Declarations

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Informed consent was obtained from all individual participants included in the study.

Conflict of Interest Yaqi Li declares that there is no conflict of interest. Tao Yu declares that there is no conflict of interest. Jiyang Li declares that there is no conflict of interest. Dezhao Kong declares that there is no conflict of interest. Qiaoqiao Shi declares that there is no conflict of interest. Chang Liu declares that there is no conflict of interest. Chunwang Dong declares that there is no conflict of interest.

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