J Fluoresc (2011) 21:1525–1530
DOI 10.1007/s10895-011-0840-4

ORIGINAL PAPER

Detection of *Staphylococcus aureus* Carrying the Gene for Toxic Shock Syndrome Toxin 1 by Quantum-Dot-Probe Complexes

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Received: 28 October 2010 / Accepted: 10 January 2011 / Published online: 28 January 2011

Abstract In this study, a high-sensitive and high-specific method to detect the toxic shock syndrome toxin-1 (TSST-1)-producing *Staphylococcus aureus* was developed based on quantum dot (QD) and oligonucleotide probe complexes. *S. aureus* carrying *tst* gene which is responsible for the production of TSST-1 were detected based on fluorescence resonance energy transfer (FRET) occurring between CdSe/ZnS QD donors and black hole quencher (BHQ) acceptors. QD-DNA probe was prepared by conjugating the carboxyl-modified QD and the amino-modified DNA with the EDC. Photoluminescence (PL) quenching was achieved through FRET after the addition of BHQ-DNA which was attached to *tst* gene probe by match sequence hybridization. The PL recovery was detected in the presence of target DNA by BHQ-DNA detached from QD-DNA probe because of the different affinities. In contrast, mismatch oligonucleotides and DNAs of other bacteria did not contribute to fluorescence intensity recovery, which exhibits the higher selectivity of the biosensor. The experimental results showed clearly that the intensity of recovered QD PL is linear to the concentration of target DNA within the range of 0.2–1.2 μM and the detection limit was 0.2 μM.

Keywords Biosensor · Fluorescence resonance energy transfer · *Staphylococcus aureus* · *tst* gene · Quantum dots

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Introduction

Staphylococcus aureus is an important pathogen causing a series of diseases such as pneumonia, mastitis, food poisoning, toxic shock syndrome, and other diseases in human and animal. Pathogenesis responsible for the symptoms and infections caused by *S. aureus* is attributed to the effect of toxins and enzymes encoded by different genes. These virulence factors include enterotoxins (SEA-SEM), exfoliative toxins (ETA and ETB), toxic shock syndrome toxin-1 (TSST-1), staphylolysin, coagulase and etc. [1]. TSST-1 plays a central role in the pathogenesis of toxic shock syndrome (TSS) which is characterized by fever, hypotension or shock, skin rash, desquamation of both hands and feet skin, and multi-organ involvement [2]. The *tst* gene is present in up to 70% of the *S.aureus* strains isolated from patients suffering from TSS. Therefore, the detection of TSST-1-producing *S. aureus* strains being recognized as contaminants of clinical samples is important not only in studying pathogenesis contributed to *S. aureus* infection but also in screening virulent strains at microbiology laboratory. A large number of detection methods have been proposed for TSST-1, including immunological methods [3], reversed passive latex agglutination (RPLA) [4] and polymerase chain reaction [5]. However, the immunological methods are time consuming and depending on the quantity of toxin produced due to culture conditions [6], as well as yielding false-positive results because of cross-reactions [7, 8]. Also, the method of PCR is easy polluted during the operation and necessitates extensive laboratory work [9]. Although many methods to detect TSST-1- producing *S. aureus* have been developed based on the use of PCR products or probes for the *tst* gene [10,

11], these approaches may be designed as identification tools rather than detections and without low detection limits. Hence, it is desirable to develop a more stable, simpler and more sensitive and selective method for the detection of TSST-1- producing *S. aureus*.

Semiconductor QDs have shown great promise as fluorescent probes for biosensing applications due to their unique properties of high quantum yield, size-dependent, narrow tunable emission and photochemical stability compared to common organic fluorophores [12, 13]. CdSe especial CdSe/ZnS core/shell QDs have been the most studied nanomaterials suitable for biological signal transduction [14] or fluorescent imaging [15] because they show the advantage of full visible-range absorption spectrum, long life time and high-intensity light emission. Fluorescence resonance energy transfer (FRET) is a wide used technique that can be applied for high-sensitivity detection of various biomolecules such as enzymes, proteins and nucleic acids based on energy transfer from a donor to an acceptor [16–18]. FRET occurs only when the energy emission bands of donors and energy absorption bands of acceptors overlap with each other. Consequently, QDs highlight their potential as excellent energy donors in FRET-based sensors benefit from their properties of high quantum yields and tunable narrow emission bands [19]. Recently, there were a number of biosensing strategies that rely upon the quenching of light emission from QDs by organic quencher molecules [20] or gold nanoparticles [21], in which FRET occurred between QDs and quenchers. QD FRET-based biosensors have many advantages over antibodies because they are easier to control, less costly, more stable against denaturation and highly sensitive according to the distance between donors and acceptors.

In this work, we employed a new approach to detect *tst*-harboring *S. aureus* based on fluorescence quenching and FRET (Fig. 1). Here carboxyl-modified CdSe/ZnS QDs were used, in which exhibited excellent stability in most buffer solutions over a wide range of pH as energy donors and allowed for the formation of QD-DNA conjugates with amino-modified DNA by the dehydration of EDC. When the QD-DNA conjugates were bond to match DNAs labeled with a BHQ (530 nm absorption), the fluorescence of the QDs (530 nm emission) was quenched apparently because the energy could transfer from QD to BHQ. As soon as the addition of the *tst* to this system, a noticeable photoluminescence (PL) recovery of the QDs was displayed due to the replacement from BHQ-DNA to target DNA. The detection of the total DNAs containing *tst* gene derived from the pathogen by this sensing system was also well demonstrated. This strategy could provide a rapid, high-sensitive and specific method to detect TSST-1-producing *S. aureus* and has great significance in pathogen detection, medical research, clinical diagnosis and treatment.

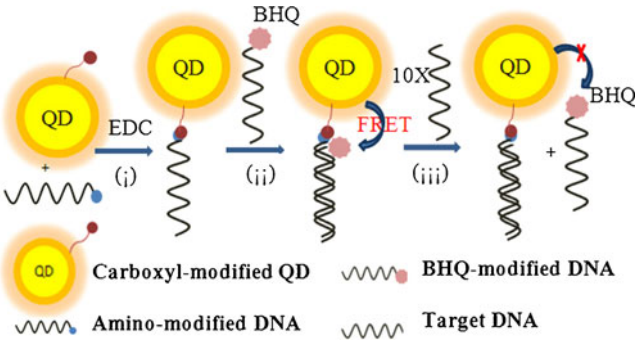

Fig. 1 Experimental scheme for detecting *S. aureus* carrying *tst* gene with QD-DNA probe complexes based on FRET. (i) amino-modified DNA are linked to carboxyl-modified CdSe–ZnS QD, (ii) addition of 1 equivalent of the BHQ-DNA to the QD-DNA to yield the hybrid and the fluorescence of QD is quenched, (iii) addition of ten equivalents of the target DNA to the hybrid to detach the BHQ-DNA from the QD-DNA accompanying by a recovery of the emission of QD. For clarity, only one carboxyl group is shown on the surface of QD

Materials and Methods

Materials

530 nm carboxyl-modified CdSe/ZnS QDs were purchased from Ocean NanoTech Company (America). At least 120 carboxyl groups were on the surface of each quantum dot (Product information). As the species-specific *tst* gene of *S. aureus* was obtained through Gene Bank (AY074881), the oligonucleotide probe was prepared following a previously described method [22]. 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC) and modified and unmodified oligonucleotides (Table 1) used in these experiments were synthesized by the Sangon Biotechnology Company (Shanghai, China). Other reagents were purchased from Peng Cheng Company (Changsha, China). Activation buffer (borate buffer, 50 mM, pH 7.4), coupling buffer (borate buffer, 50 mM, pH 8.3) and the sodium phosphate buffer (PBS, 10 mM, pH 7.0) were prepared with ultrapure Mill-Q water (electric resistivity 18 MΩ cm^{-1}). All the water used in this experiment was purified with the Millipore-Q system (Millipore Inc. America). *S. aureus* strains originated from Xiangya Hopital of Central South University (Changsha, China). Other reference bacteria strains were kept in our laboratory.

Instruments

Fluorescence spectra were measured from a LS55 luminescence spectrometer (PerkinElmer, U.K.) at 28 °C. The fluorescence signals were recorded from 380 nm to 680 nm when the solutions were excited at 320 nm. Excitation and emission spectrometer slits were both set for 10.0 nm. The

Table 1 Oligonucleotide sequences of probe[a], target and mismatch DNAs[b]

Oligonucleotide	Sequence(5′–3′)
Amino-DNA	NH_2-TAACTCAAATACATGGATTATATCGTTC
BHQ-DNA	GAACGATATAATCCATGTATTTGAGTTA-BHQ
Target	GAACGATATAATCCATGTATTTGAGTTA
Two bases mismatch	GAACGGTATAATCCATGTATATGAGTTA
Twelve bases mismatch	GAAGTATCGAATGATTGTACATTCGTTC

[a] Note that the selected nucleotide sequence for probe is the 530–557th nucleotide sequence of *tst* gene

[b] The underlined bases indicate the base mismatch

scan rate was 1500 nm s^{-1}. Purification was achieved by using a centrifuge (Hettich Universal 32R, UK).UV spectrophotometer used for absorbance detection of DNA at 260 nm or 280 nm was purchased from Tianmei Techcomp (Shanghai, China).The purified DNA stocks of bacteria strains used in this study were prepared by TIANamp Bacteria DNA Kit from Tiangen Biotech Company (Beijing, China).

Preparation of QDs-DNA Probe Complexes

Single-stranded DNA was linked to CdSe–ZnS QDs. To achieve this, a 5′-amino-modified DNA was first synthesized by introduction of the C6 amidite (Sangon). The carboxyl functionalised QDs were treated with EDC (2 mg mL^{-1}), HCl and borate buffer (50 mM, pH 7.4) for 30 min at room temperature in a 1.5 mL capacity tube. Then borate buffer (50 mM, pH 8.3) was added to the QDs solution, followed by appropriate volume of 5′-aminohexyl-substituted DNA. This solution was mixed for 2 h at room temperature and then stored overnight (4 °C). To remove excess EDC and unconjugated 5′amino-modified DNA, purification was achieved by ultracentrifugation (80 min, 34000 g) at 4 °C and the supernatant was discarded [21]. After it was redispersed to a concentration of 0.12 μM in PBS solution (10 mM, pH 7.0), the QD–DNA conjugate was found to be thermally unstable and stable when stored at 4 °C. Since FRET efficiency was mainly affected by the ratio of amino-modified DNA and carboxyl-modified QDs, six different ratios (10:1, 20:1, 30:1, 40:1, 50:1, 60:1) were examined by PL spectroscopy at the same conditions, respectively. Finally, the 3′-BHQ-modified complementary DNA was added to the QD-DNA in a concentration ratio of 1:1 and the BHQ quenched the fluorescence of the QDs in 1.5 h.

Detection of *tst* Gene and Mismatch DNA and Total DNA of Bacteria

As the probe was formed, ten equivalents (1.2 μM) of target oligonucleotides were added to this solution, resulting in recovery of the emission in 1 h and the target DNA was measured by monitoring the variation in PL intensity. Six different concentrations of the *tst* from 0.2 μM to 1.2 μM were tested. In addition, two different mismatch DNAs (as shown in Table 1) were tested as control experiments at the same conditions. Finally, four different kinds of total DNA obtained from different bacteria (*S. aureus*, *E. coli, S. epidermidis, S. paratyphoid A*) by using TIANamp Bacteria DNA Kit were tested. Bacteria strains were cultivated in a 100 mL flask with 50 mL of Luria Bertani (LB) medium (beef extract 1%, yeast extract 0.5%, NaCl 1%, pH 7.4) at 37 °C for 24 h, with shaking at 100 rpm. The concentrations of mismatch DNA and total DNA were the same with that of target DNA.

Results and Discussion

Effect of the Ratio of DNA to QD on FRET Efficiency

As reported before [23], the ratio of acceptor to donor was a critically important factor for FRET efficiency. The ratio was determined by that of amino modified DNA to carboxyl-modified QD when the BHQ-DNA concentration was kept at constant. The high ratio of amino-DNA to carboxyl-modified QD was benefit to the FRET efficiency due to the high concentration of QD-DNA bioconjugates. Since the average number of carboxyl groups on the surface of the quantum dot was known from the product information, six different ratios of amino modified DNA to carboxyl modified QD (10:1, 20:1, 30:1, 40:1, 50:1, 60:1) were measured. QD was kept at 6 pmol for formation of QD-DNA conjugation at these different ratios. After the QD-DNA conjugations were prepared and purified, they were resuspended in 400 μl PBS solution (10 mM, pH7.0), and 0.18 nmol BHQ-DNA and 1.8 nmol target DNA were added in again. The final total volume of the mixed solution in every tube was kept at 400 μl. Various DNA/QD ratios tests (corresponding amino-DNA were 60 pmol, 120 pmol, 180 pmol, 240 pmol, 300 pmol, 360 pmol) were taken under the same conditions. The fluorescence spectra were detected and analyzed after the whole reaction. Figure 2 showed the FRET efficiency calculated from the Eq. 1 [24],

$$E = 1 - F_{DA}/F_D \quad (1)$$

where F_{DA} is the PL intensity of the carboxyl-modified QD at 530 nm in the presence of the acceptor BHQ, and F_D is the PL intensity of the carboxyl-modified QD in its absence, respectively.

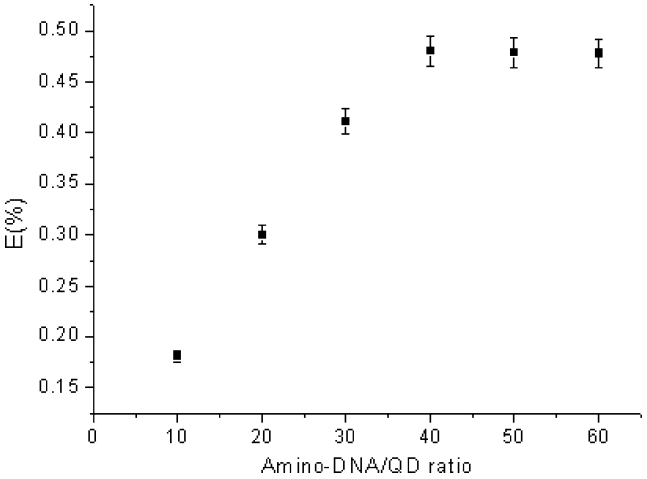

Fig. 2 The FRET efficiency of the QD complexes at various ratios (10–60) of DNA (amino-modified) to QD (carboxyl-modified).FRET efficiency is calculated from Eq. 1. When the ratio is at 40, it has a highest FRET efficiency of 48%. Error bars SD (n=3)

As can be seen from the Fig. 2, the value of E increased coupling with the increase of the DNA/QD ratio till almost was constant at the ratio of 40:1, demonstrating that the reaction reached its equilibrium. This equilibrium ratio at 40:1 indicated that the steric resistance resulting from the formation of DNA/QD conjugates prevented more DNAs from locating to QD surface since theoretically each QD with 120 carboxyl groups on the surface could conjugate about 120 amino-modified DNAs. Moreover, the EDC was not so efficient at conjugation between the amino-DNA and carboxyl-QD. The FRET efficiency of this complex reached nearly 48% with the DNA/QD ratio at 40:1, while a control experiment was carried out to see whether DNA without BHQ label could affect the QD fluorescence. Under the same conditions as described above, the quenching of the QD fluorescence was less than 10% (data not shown). Therefore, the ratio of 40:1 was used in latter work.

Specificity Shown by the Detection of Mismatch DNA

The ability of the QD-DNA complexes to detect target DNA was tested in the presence of complementary and noncomplementary with two and twelve mismatch bases (Fig. 3). In this section, the target DNA and mismatch DNA were tested at the same conditions with the concentration of 0.48 nmol in 400 μl PBS solution. Control experiment was carried out with the absence of target DNA. As shown in Fig. 3, significant recovery of QD fluorescence at 530 nm (Fig. 3(d)) was observed with complementary DNAs. The FRET efficiency decreased as increased in the recovery of the QD PL, resulting from the addition of the target DNA. This suggested that the target DNA bind to the QD-DNA by competing with the BHQ-DNA, and this resulted in an increase in the distance of the BHQ dye from the QD surface.

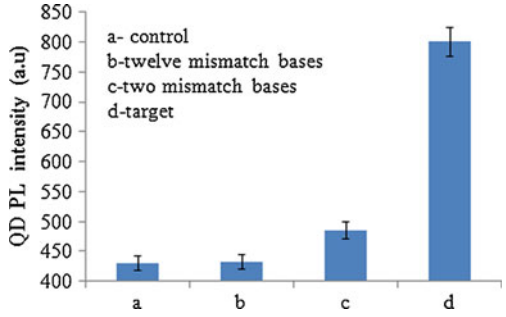

Fig. 3 The different recovery of PL intensity of QD with the addition of target DNAs and mismatch DNAs. **a** Initial quenched QD PL without DNA, **b-c** weak recovery of QD fluorescence with different numbers of mismatch bases of DNAs, **d** apparent recovery of QD fluorescence at 530 nm after addition of target DNAs. The concentrations of QD, mismatch DNAs and target DNA are 30 nM, 1.2 μM and 1.2 μM in PBS solution (10 mM, pH7.0), respectively. Error bars SD (n=3)

The QD fluorescence intensity was nearly to that of initially quenched QD fluorescence on addition of two and twelve mismatch bases of DNAs compared with the target DNA (Fig. 3(a–d)). It was indicated that this detection is high sequence-specific and discriminable with mismatch DNAs.

Detection of Target DNA Oligonucleotides

The detection of *tst* gene (target DNA) by hybridization of QD-DNA conjugation and BHQ-DNA in the range of 0.2–1.2 μM was carried out using 3 nM QD-DNA complexes (Fig. 4). In this test, six different concentrations of target DNAs (0.2 μM, 0.4 μM, 0.6 μM, 0.8 μM, 1.0 μM and 1.2 μM) in 400 μl PBS buffer solutions were studied with 0.048 nmol BHQ-DNA at the same conditions, respectively. When target DNAs were added to the complex of QD-DNA and BHQ-DNA in PBS solution, significant recovery of QD fluorescence was monitored since BHQ-DNA was detached from the QD-DNA and thus FRET efficiency decreased. It could be explained that the target DNA had a stronger affinity to the QD-DNA compared to the BHQ-DNA for the different level of the concentration of target DNA and BHQ-DNA (10:1) [21].The higher the concentration of target DNA is, the higher its affinity with probe is when the BHQ-DNA is kept at constant. It was found that FRET efficiency can be effectively suppressed by the detachment of quenchers from QD-DNA. As presented in Fig. 4, there was an apparent recovery of QD fluorescence at 530 nm with the addition of target DNA, and the intensity of recovered QD fluorescence showed nearly linear increase to the concentration of target DNA. More importantly, fluorescence changes were very sensitive to the addition of very small volume of the targets, which suggested that this method can detect low quantitivity of *tst* gene and its detection limit was up to ~200 nM.

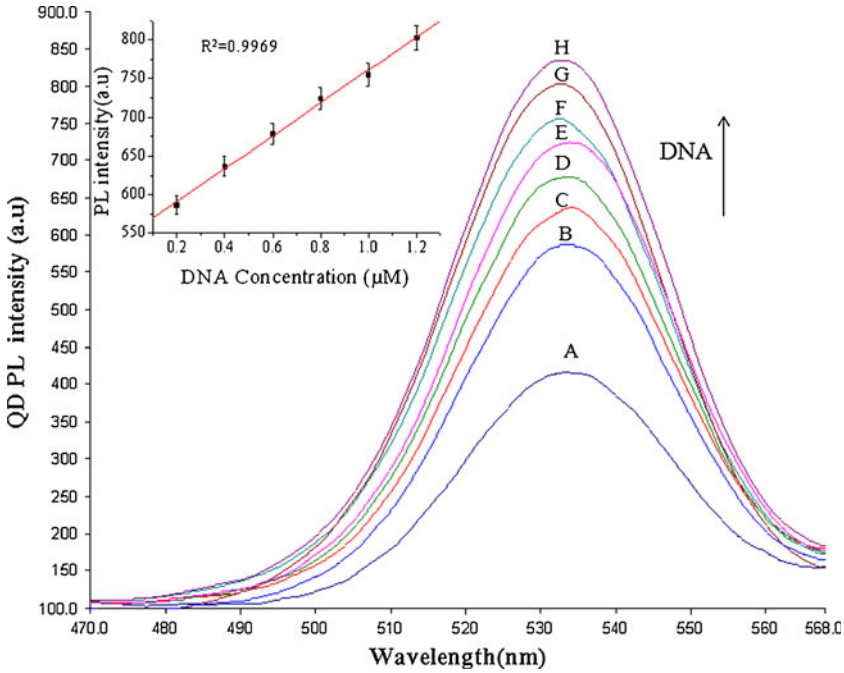

Fig. 4 The detection of target DNA at different concentrations. Fluorescence spectra show the PL intensity variation of QD-DNA probe complexes H) without and **a** with quenchers. The target DNA concentrations are varied at **b** 0.2 μM, **c** 0.4 μM, **d** 0.6 μM, **e** 0.8 μM, **f** 1.0 μM and **g** 1.2 μM. (The inserted figure shows the increase in fluorescence intensity is proportional to concentration of target DNA). Error bars SD (n=3)

Detection of the *tst*-Harboring *S. aureus*

To further detect *S. aureus* carrying *tst* gene by this method, total DNAs of four different strains were extracted using TIANamp Bacteria DNA extraction kits, individually. UV absorbance of the DNAs at 260/280 nm was measured to determine their concentration and purity. The extracted DNAs from each bacteria strain were diluted in ultrapure water to 1.2 μM for detection at the same conditions. As shown in Fig. 5, fluorescence signal was low (Fig. 5b) before the DNAs were added because of the high quenching efficiency of BHQ. When the DNAs of other three bacteria were added into the system, respectively, the PL intensities of the quenched QDs were still low (Fig. 5c–e). By contrast, the degree of recovery was higher than that caused by other three bacteria and the intensity of recovered fluorescence (Fig. 5f) was close to that of original unquenched QDs as soon as the DNAs of *S. aureus* was added to it. Therefore, it could be concluded that the increasing in PL intensity comes only from the reaction with DNA of *S. aureus*, and the system possesses high selectivity. Furthermore, this QD/DNA probe based on FRET could be used to detect not only *S. aureus* carrying *tst* gene, but also other bacteria carrying completely similar pathogenicity island genes.

Conclusion

We have successfully prepared quantum-dot-probe complexes to detect specific *tst*-harboring *S. aureus* which produce toxic shock syndrome toxin-1 to host. This method is clearly simple, rapid, sensitive and convenient by using the fluorescence quenching caused by the CdSe/ZnS quantum dots and BHQ. The quenching of QD fluorescence was found to be reversed on addition of target DNA whereas the reverse was not shown upon the additions of DNAs of other bacteria, which implied that the probe has strong specificity to *S. aureus*. This new assembled FRET

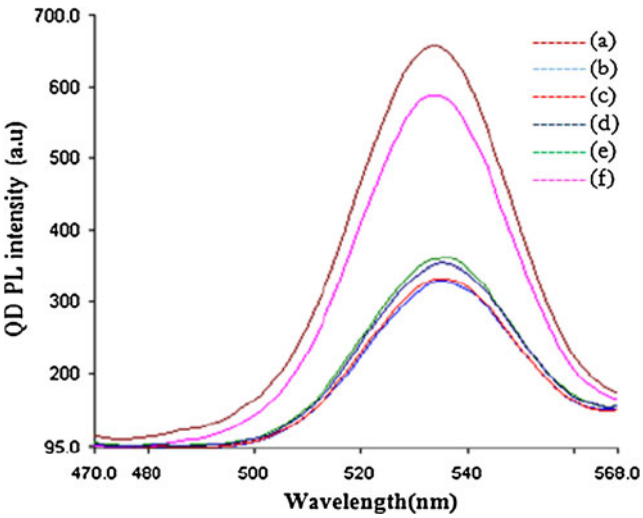

Fig. 5 Specific detection of *tst* gene from *S. aureus* strains compared to other bacteria strains. Changes in fluorescence spectra of QD-DNA probe complexes (**a**) without and (**b**) with quenchers. From (**c**) to (**f**) present the PL intensity variation in the presence of *S. paratyphoid A*, *E. coli*, *S. epidermidis* and *S. aureus*, respectively. The concentration of these four DNAs extracted from these four bacteria strains is at 1.2 μM, respectively

based probe can be used to detect target DNA at 0.2 μM and shows that the increase in fluorescence intensity is proportional to target DNA ranged from 0.2 to 1.2 μM. Since virulent *S. aureus* cause an increasing worldwide problem both in the hospital and in the community, the availability of this method for the identification and detection of *tst*-harboring *S. aureus* may be applied for the clinical diagnosis. In addition, the strategy of this quencher-bearing oligonucleotide could be extended to other DNA, virus or protein sensing applications. Our future researches will mainly focus on optimizing the reaction conditions to improve the detection sensitivity.

Acknowledgements We would like to thank National Natural Science Foundation (30770570) and 863 Program of Ministry of Science and Technology (2007AA062Z403) for the financial support.

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