Chinese Chemical Letters 32 (2021) 791–795

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Communication

Aptamer-quantum dots and teicoplanin-gold nanoparticles constructed FRET sensor for sensitive detection of *Staphylococcus aureus*

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ARTICLE INFO

Article history:
Received 27 April 2020
Received in revised form 4 June 2020
Accepted 8 July 2020
Available online 9 July 2020

Keywords:
Fluorescence resonance energy transfer (FRET)
Staphylococcus aureus
Aptamer
Quantum dot
Teicoplanin
Gold nanoparticles

ABSTRACT

The detection of bacterial pathogen such as *Staphylococcus aureus* (*S. aureus*) is essential for the regulation of food hygiene and disease diagnosis. Herein, we developed a simple one-step fluorescence resonance energy transfer (FRET)-based sensor for specific and sensitive detection of *S. aureus* in food and serum samples, in which aptamer-modified quantum dots (aptamer-QDs) was employed as the energy donor and antibiotic of teicoplanin functionalized-gold nanoparticles (Teico-AuNPs) was chosen as the energy acceptor. Within 1 h, the FRET-based sensor showed a linear range of from 10 cfu/mL to 5×10^8 cfu/mL, with the low limit of detection (LOD, 2 cfu/mL) for *S. aureus* in buffer. When further applied to assay *S. aureus* in real samples, the FRET-based sensor showed good recoveries ranging from 84.5% to 110.0%, with relative standard derivations (RSDs) of 0.01%–0.44% and a LOD of 100 cfu/mL in milk, orange juice and human serum.

Staphylococcus aureus (*S. aureus*), a Gram-positive and round-shaped bacterium, is the top common pathogen causing serious food poisoning and infectious diseases by producing enterotoxins or invading organism [1–6]. Usually, *S. aureus* is widespread in the environment (air, water, dust, human or animal skins, soil *etc.*) but typically occurs at low levels, which makes the task of detecting *S. aureus* challenging [7]. What is more, *S. aureus* can be readily transmitted between humans and animals [4,8]. Given the easy transmission and great health threat of *S. aureus*, it is of great interest and significance to establish a rapid, sensitive, specific, simple and low-cost approach to detect *S. aureus* for food hygiene regulation and disease diagnosis in today's health care [3,4]. Due to the reliability of traditional colony culture [9,10] and high sensitivity of polymerase chain reaction (PCR) [11–13], the two methods were widely applied to assay *S. aureus* in real samples. However, several limitations including time-consuming and low-sensitivity for culture method (at least 2 days) [4,14] and false-positive results for PCR [15], making them not ideal method for rapid and onsite analysis of *S. aureus* timely. Recently, instrumental analysis was used to improve the speed and sensitivity for *S. aureus* detection [16–18], suffering the disadvantages such as expensive instruments and laborious sample pretreatment. To overcome the drawbacks of above-mentioned methods, biosensor-based strategies have been widely established by employing various recognition molecules (antibody, peptide, aptamer, *etc.*) in combination with different transduction signals such as fluorescence [19–22], electronic [11,23], surface plasmon resonance [24,25], Raman scattering [26–28], photoacoustic [1].

Due to its merits of simple, rapid, sensitive for assay, fluorescence resonance energy transfer (FRET) technique has been intensively applied in sensing various targets, including *S. aureus* [29,30]. Choosing appropriate energy donor-acceptor pairs could improve the analytical performance of FRET-based sensor [31]. Previously, we developed a FRET sensor to detect *S. aureus* employing vancomycin stabilized gold nanoclusters (AuNCs) as the energy donor and aptamer-labeled gold nanoparticles as the energy acceptor [19]. However, the fluorescence quenching efficiency ($\eta_{max} < 21\%$) was relatively lower, which was attributed to the poor spectrum overlap between the donor (emission spectrum) and acceptor (absorption spectrum). Quantum dots (QDs) with excellent fluorescent property, are widely used for biological sensing and imaging [32]. QDs with narrow emission and broad absorption spectra made them excellent FRET energy donors for constructing various chem/biosensors [33,34]. As a broad-spectrum antibiotic active against Gram-positive bacteria including *S. aureus* [35,36], teicoplanin had the similar chemical

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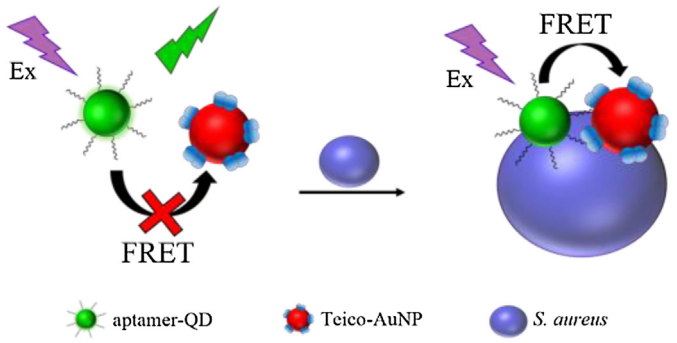
https://doi.org/10.1016/j.cclet.2020.07.020



Scheme 1. Schematic illustration of FRET-based sensor for *S. aureus* by employing aptamer-QDs (donor) and Teico-AuNPs (acceptor).

structure with vancomycin except the an eight-carbon unit long alkane chain, and could be employed as reducing agent and stabilizer to generate teicoplanin-functionalized AuNPs (Teico-AuNPs) according to the published work [37].

Inspired by the prior study, a further new FRET-based sensor by employing aptamer-QDs (energy donor) with the emission spectrum mostly overlapping with the absorption spectrum of the Teico-AuNPs (energy acceptor) were developed to enhance the assay sensitivity and fluorescence quenching efficiency for rapid, ultrasensitive and specific determination of *S. aureus* (Scheme 1). In the presence of target, the aptamers and teicoplanin bound to *S. aureus* simultaneously, making the energy donor (aptamer-QDs) and acceptor (Teico-AuNPs) dramatically close to each other and subsequently the FRET turned "on". Due to the high fluorescence quenching efficiency of as-prepared FRET sensor, *S. aureus* in milk, orange juice, and human serum sample could be detected sensitively and rapidly in one-step. The developed FRET-based sensor with dual recognition by employing aptamer-QDs and Teico-AuNPs enables rapid, sensitive and specific bacterial detection for food safety and public health.

In this study, we focused to improve assay sensitivity based on enhanced fluorescent quenching efficiency by employing QDs as the energy donor and AuNPs as the energy acceptor. The aptamer specific to *S. aureus* was conjugated with QDs to obtain the donor of aptamer-QDs according the previously published procedures [38], whose preparation was shown in the Supporting information. The dynamic light scattering (DLS) dimeter of aptamer-QDs increased from 27 nm (streptavidin-QDs) to 33 nm (Fig. 1A), and aptamer-QDs moved faster than that with streptavidin-QDs in agarose gel due to the binding of DNA aptamer to the streptavidin-QDs (Fig. 1B). The emission spectrum of streptavidin-QDs before and after conjugating with biotin-aptamer (aptamer-QDs) were almost overlapped (Fig. 1C), showing good fluorescence quality of aptamer-QDs (the donor). To improve the recognition effect of the FRET-based sensor to *S. aureus*, another recognition molecule of teicoplanin was employed as reducer and stabilizer to prepare Teico-AuNPs (the acceptor). Teico-AuNPs were synthesized from $HAuCl_4$ in one step with optimum conditions of W_{HAuCl4}/W_{Teico} = 4:1 and incubation at 60 °C for 15 min at pH 12 (Fig. S1 in Supporting information). Teico-AuNPs with color of wine red had a typical absorption spectrum with peak at 520 nm (Fig. 1D). The morphology of Teico-AuNPs was observed by transmission electron microscop (TEM) imaging, showing that Teico-AuNPs was uniform monodisperse spherical nanoparticle with a diameter of 13.5 ± 1.2 nm (Fig. 1E). The zeta potential of Teico-AuNPs was about −51.7 ± 0.4 mV (Fig. 1F), providing enough surface charge to stabilize the nanoprobes. All the above results confirmed the success in synthesis of Teico-AuNPs. The bioactivity of Teico-AuNPs for the inhibition to bacteria (*S. aureus*) was obtained by counting the number of colonies on Petri dishes. Teico-AuNPs exhibited excellent antibiotic activity to *S. aureus* (Fig. 1G), demonstrating that Teico-AuNPs could bind with *S. aureus*, providing the basis for the FRET-based sensor strategy developed in this work. The

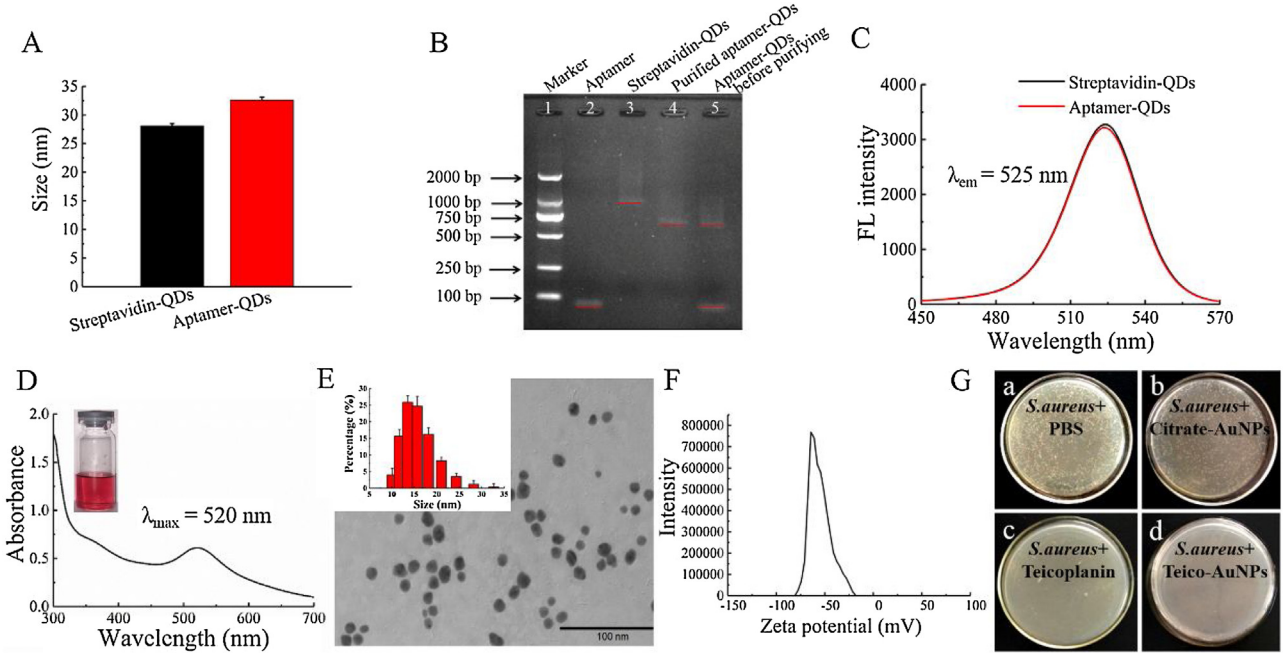


Fig. 1. (A) DLS size distribution, (B) digital image of agarose gel electrophoresis, and (C) fluorescent spectra of streptavidin-QDs before and after conjugating with aptamer. (D) The absorption spectrum of Teico-AuNPs (The inset is a digital photo), (E) TEM photo of Teico-AuNPs (The inset is size distribution), and (F) zeta potential of Teico-AuNPs. (G) Digital photos of *S. aureus* treated with different solutions for 2 h and then cultured on individual Petri dishes overnight.

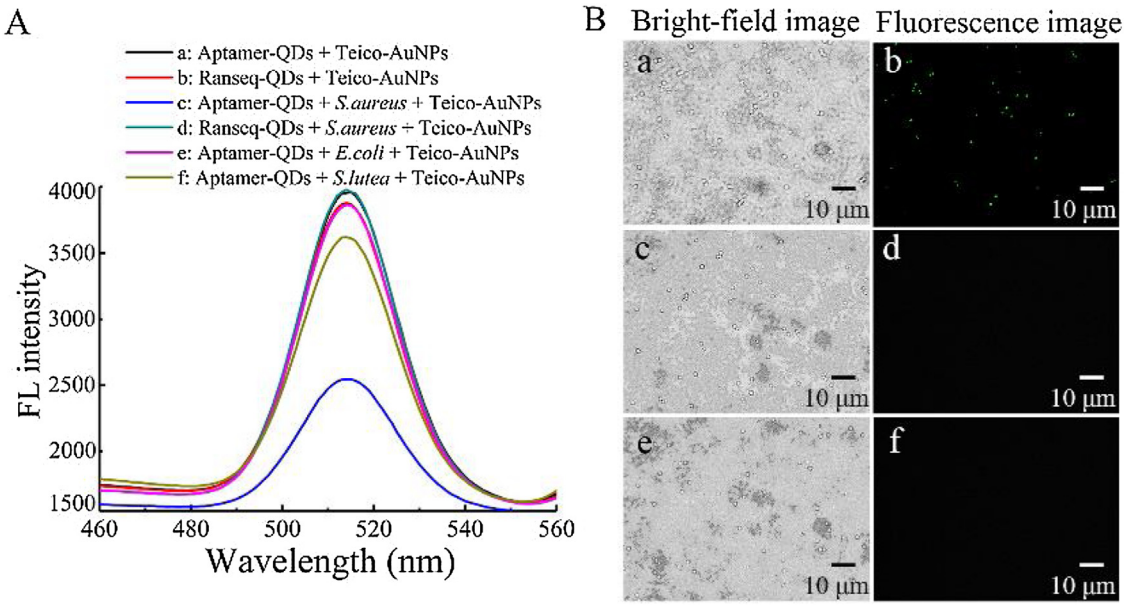


Fig. 2. (A) Fluorescence spectra of the aptamer-QDs after mixing with Teico-AuNPs, bacteria, or both of them. (B) Fluorescence microscope images of *S. aureus* after incubation with aptamer-QDs only (a, b), with the mixture of aptamer-QDs and Teico-AuNPs (c, d), and with the mixture of RanSeq-QDs and Teico-AuNPs (e, f) simultaneously.

aptamer-QDs could be used after balanced for 48 h once they were produced (Fig. S2A in Supporting information). The absorption value at 520 nm of Teico-AuNPs showed negligible change after 30-days storage at room temperature (RT) (Fig. S2B in Supporting information) and in different buffers after incubating for 2 h (Fig. S2C in Supporting information). The above results demonstrated that both aptamer-QDs donor and Teico-AuNPs acceptor had good stability. The emission spectrum of aptamer-QDs and the absorbance spectrum of Teico-AuNPs were overlapped (Fig. S2D in Supporting information), suggesting that aptamer-QDs and Teico-AuNPs were a nice pair of FRET donor/acceptor.

The FRET-based sensor for *S. aureus* with aptamer-QDs and Teico-AuNPs was illustrated in Scheme 1. The FRET-based sensor will turn "on" when there was in the presence of *S. aureus* due to simultaneous recognition of aptamer-QDs and Teico-AuNPs to the target *S. aureus*, making the donor and acceptor dramatically close to each other. The strong fluorescence of aptamer-QDs donor was obviously quenched by Teico-AuNPs acceptor with the presence of *S. aureus* with fluorescence quenching efficiency η of about 57.52% (Fig. 2A) (η was determined by the equation $\eta = (F_0–F)/F_0 \times 100\%$, where F_0 and F were the fluorescence intensity for the FRET-based sensor in the absence and presence of *S. aureus* or other bacteria, respectively) [19]. This high fluorescence quenching efficiency could be attributed to the maximum overlapped spectrum between the emission spectrum of aptamer-QDs (donor) and the absorbance spectrum of Teico-AuNPs (acceptor) (Fig. S2D) when they bound to the target simultaneously. However, when a random DNA sequence (RanSeq) replaced the aptamer specific to *S. aureus* or no target *S. aureus* (such as *S. lutea, E. coli*) was present, there was only a slight fluorescence intensity decrease, indicating the FRET-based sensor can specifically detect target bacteria *S. aureus*. Meanwhile, the phenomenon of fluorescence quenching could be visually observed in a fluorescence microscope. Compared with bright green fluorescence dots (b) around the site where *S. aureus* located (a) when only incubated with aptamer-QDs, the fluorescence image (d) exhibited subdued green fluorescence dots on the same sites where *S. aureus* located (a) when treated with aptamer-QDs/Teico-AuNPs mixture (Fig. 2B). While no green fluorescence dots (f) on the same sites where the *S. aureus* located (e) when incubated with the mixture of RanSeq-QDs and Teico-AuNPs (e and f). For specificity test, several other different bacteria were analyzed. As showed in Fig. S3 (Supporting information), all the bacteria except the target *S. aureus* can hardly cause obvious fluorescence intensity change ΔF ($\Delta F = F_0–F$), demonstrating the FRET-based sensor has good specificity for *S. aureus*.

After optimized the assay conditions (Fig. S4 in Supporting information), the linear range and limit of detection (LOD) of the FRET-based sensor for *S. aureus* were investigated. The linear regression equation [$\Delta F/F_0 = 0.1133\log_{10}N - 0.0966$ ($R = 0.9988$)] was obtained by plotting $\Delta F/F_0$ *versus* logarithm of the *S. aureus* concentration (from 10 cfu/mL to 5×10^8 cfu/mL) ($\Delta F = F_0–F$, where F_0 and F are the fluorescence intensity in the absence and presence of *S. aureus*, respectively; N stands for *S. aureus* quantity expressed as cfu/mL) (Fig. 3). The LOD of the FRET-based sensor was 2 cfu/mL, determined by the equation LOD = $3S/K$ (where S is the standard deviation of the blank samples (n = 10) and K is the slope of the calibration curve). Compared with our previous work [19], the FRET-based sensor for *S. aureus* detection in this study showed

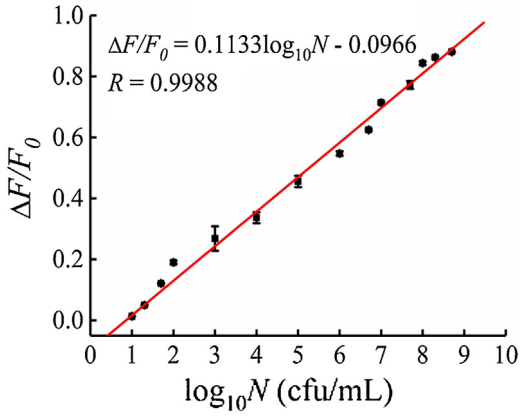


Fig. 3. The calibration curve of the changed fluorescence intensity ($\Delta F/F_0$) on the logarithm of *S. aureus* concentration.

wider detection range (from 10 cfu/mL to 5×10^8 cfu/mL) and lower LOD (2 cfu/mL), which was attributed to the improved fluorescence quenching efficiency (highest to 88.09%) by great overlapped spectrum between the emission spectrum of aptamer-QDs (the donor) and the absorbance spectrum of Teico-AuNPs (the acceptor). To show the superiority of aptamer-QDs based donor, the aptamer-FAM was employed as donor to construct FRET sensor for *S. aureus*. There was almost no any signal change when the *S. aureus* concentration was as low to 30 cfu/mL or as high to 1×10^6 cfu/mL (Fig. S5 in Supporting information), demonstrating the performance (detection range and LOD) of aptamer-FAM donor-based FRET sensor is inferior to that of aptamer-QDs donor-based FRET sensor.

In order to verify the applicability of the proposed FRET-based sensor in real samples, three real samples (milk, orange juice and human serum samples from healthy volunteers) spiked with different concentrations of *S. aureus* were chosen as the model. The method was performed after the samples were properly diluted without future sample pretreatment, and the interference of the real samples (the background signals from the real samples not spiking any *S. aureus*) to the FRET-based sensor was also explored. Ten-fold dilution of milk, juice and human serum samples produced comparable background signals with that of BBS buffer (blank) (Fig. 4A). The 10-fold dilution real samples spiked with different concentrations of *S. aureus* were subjected to analysis with FRET-based sensor. The recoveries ranged from 84.5% to 110.0% with relative standard derivations (RSDs) of 0.01%–0.44% (Table 1), suggesting that the FRET-based sensor can be applied to detection of *S. aureus* in real samples. The LODs of the FRET-based sensor for *S. aureus* in the milk, orange juice and human serum sample (10-dilution) were 100, 100, and 100 cfu/mL, respectively (Fig. 4B). Although diluting real samples were necessary before FRET-based sensor assay, fortunately, the operation of dilution was simple, without affecting the efficiency of the FRET-based sensor seriously. When do the same assay of *S. aureus* in real samples using FRET-based sensor composed of aptamer-FAM, the real samples were needed to dilute for more times (up to 500-fold dilution) and also LODs were much higher than that in FRET-based sensor using aptamer-QDs, showing the superiority of the aptamer-QDs based FRET sensor (Fig. S6 in Supporting information). A brief comparison of three different donors (Vancomycin-AuNCs, aptamer-QDs and FAM-aptamer) based on the FRET strategy for *S. aureus* detection was summarized in Table S2 (Supporting information).

In this study, a facile, rapid and reliable one-step FRET-based sensor by employing aptamer-QDs (the energy donor) and Teico-AuNPs (the energy acceptor) was established for specific and sensitive detection of *S. aureus*. The proposed FRET-based sensor exhibited a quite broad linear scope (from 10 cfu/mL to 5×10^8 cfu/mL) with a low LOD (2 cfu/mL) for *S. aureus* and was successfully applied in real samples (milk, orange juice and human serum). Moreover, the FRET-based sensor with dual-recognition strategy can be applied to detection of other pathogenic bacteria in future, and has the potential to be a universal platform for bacteria detection by changing the recognition molecules specific to the desired target.

Declaration of competing interest

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

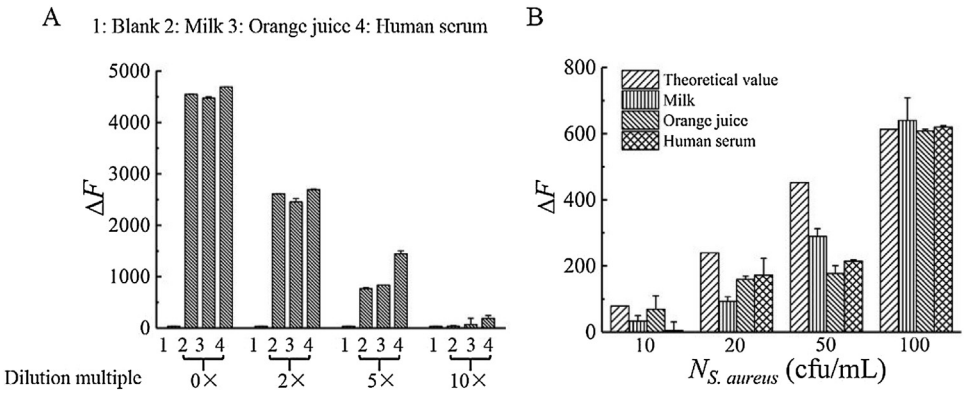


Fig. 4. (A) Fluorescence response (ΔF) of FRET-based sensor in different sample with different-fold dilution. (B) The LODs of the FRET-based sensor for *S. aureus* in milk, orange juice and human serum sample.

Table 1
Recovery of *S. aureus* in real samples with FRET-based sensor.

Sample	Added ($\log_{10}N$ (cfu/mL))	Measured ($\log_{10}N$ (cfu/mL))	Recovery (%)	RSD (%, $n = 3$)
Milk 1	2	1.69	84.50	0.07
Milk 2	4	4.33	108.25	0.01
Milk 3	6	5.93	98.83	0.08
Orange juice 1	2	2.20	110.00	0.05
Orange juice 2	4	3.74	93.50	0.27
Orange juice 3	6	6.00	100.00	0.25
Human serum 1	2	2.20	110.00	0.12
Human serum 2	4	3.85	96.25	0.01
Human serum 3	6	6.36	106.00	0.44

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 21974110, 21575118, 21976145 and 31672605), Natural Science Foundation Project of Chongqing (No. CSTC2019jcyj-msxmX0406).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cclet.2020.07.020.

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