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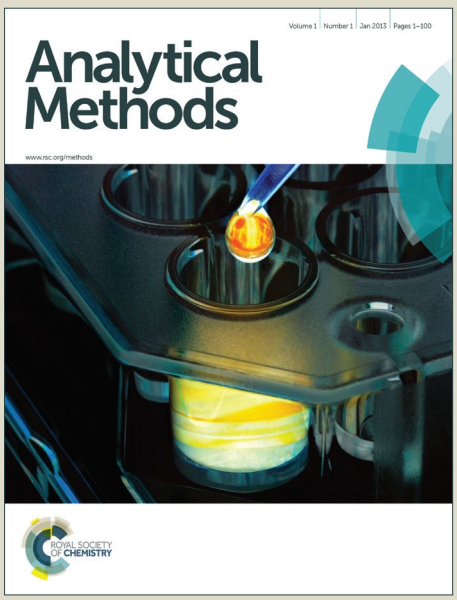

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Highly Sensitive Fluorescent Aptasensor for *Salmonella paratyphi A* Via DNase I-mediated Cyclic Signal Amplification

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Outbreaks of *Salmonella paratyphi A* (*S. paratyphi A*) infection continue to occur worldwide and have drawn close attention. A useful practical detection platform is essential to the early rapid diagnosis of the infection. In this study, a simple and cost-effective DNA aptasensor was constructed, which was composed of a designed aptamer (DA) and two short carboxyfluorescein(FAM)-modified sequences (probe 1 and probe 2) for fluorimetric determination of *S. paratyphi A*. In the absence of target, the two-FAMs aptasensor (the aptasensor) was bound to graphene oxide (GO) and the fluorescence of FAM was quenched. In the presence of target, however, the aptasensor was released from the surface of GO due to specific binding of the aptasensor to the target and a strong fluorescent signal could be detected subsequently. More importantly, the fluorescent signal could be substantially amplified by a DNase I-mediated target recycling process. Under the optimized conditions, the fluorescence intensity increased linearly with the target concentrations ranging from 1×10^2 to 1×10^{11} cells/mL with a detection limit of 1×10^2 cells/mL. These results demonstrated that this detection platform exhibited high sensitivity and specificity in the detection of *S. paratyphi A*, and it might even be a potential alternative approach for other bacteria detection.

Keywords

Aptasensor, Fluorescence intensity, *Salmonella paratyphi A*, Graphene oxide, DNase I, Target recycling.

1. Introduction

Salmonella is one of the zoonotic pathogenic bacteria in the field of public hygienics[1]. It often causes different degrees of poultry and animal infections as well as human diseases by food-borne contamination. As a timely treatment of Salmonella infection is difficult, it is required to develop a rapid detection for them[2]. As a food-borne pathogen which could be transmitted by domestic water, food, fly, cockroach and so forth[3], *S. paratyphi A* brings about a large variety of health problems, from minor manifestations such as headache, fever, anepithymia, to more serious complications of enterorrhagid, enterobrosis, myocarditis that lead to high death rate in humans. Since Salmonella was initially identified as human pathogenic bacterium in the late 19th century, testing methodologies were all based on the tests that used feces or blood from infected patients as clinical specimens[4]. However, these conventional standard detection methods have their limitations. For instance, routine inspection and bacteriological examination are time-consuming and labor intensive, generally with a week. Immunological methods are costly, require complex procedures and produce false-positive results from cross-reaction, increasing the challenge in practical application[5]. Molecular biological methods have also some disadvantages. Polymerase Chain Reaction (PCR), for instance, requires multiple touch conditions and produces some contaminations during nucleic acid sample extraction[6]. Although Amplified Fragment Length Polymorphism (AFLP) is also applied to microorganism researches[7], there are limited reports about detection of Salmonella. Therefore, it is necessary to develop a time-saving method with high sensitivity and specificity for *S. paratyphi A* detection.

Aptamers are single-stranded DNA (ssDNA) or RNA molecules obtained from nucleic acid

pool by the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) procedure in vitro, which can bind to various kinds of targets, including ions, proteins, whole cells and small molecules, with high specificity, sensitivity and affinity[8]. They have been used as biomarkers and therapeutic agents, and provide alternative element for ligand recognition in diagnosis and detection systems as compared to antibodies[9]. Meanwhile, all kinds of targets have been detected based on aptamer recently, including bacteria, adenosine triphosphate (ATP), proteins, cells, ions, molecules[10-15], etc. In short, aptamers have extensive application prospects in detection and therapy fields[16,17].

Many enzymes have been used for determination, including DNA polymerase, exonnuclease and endonuclease. DNase I is an endonuclease that can digests single- and double-stranded sequences of DNA (ssDNA and dsDNA) simultaneously and randomly. It hydrolyzes phosphodiester bonds, producing mono- and oligodeoxyribonucleotides with 5'-phosphate and 3'-OH groups. In the presence of Mg^{2+}, it cleaves each strand of dsDNA independently in a statistically random fashion. Compared to other endonucleases, it requires no specific recognition site and possesses a broad hydrolytic spectrum. Although it has some applications in many detection systems, its few works are reported in bacterial detection[18].

Nanomaterials are widely used for detection systems[19]. Graphene has attracted great attention for its remarkable properties (electronic, mechanical, and thermal)[20]. Graphite is a substance with stratified structure formed by SP^2 carbon hybridization, while graphene is just one layer of graphite. After being treated by concentrated acid or strong oxidant, graphene is oxided to form GO with hydroxy, carbonyl and epoxy groups. As a monatomic two-dimensional structure, GO has its unique features compared to other quenching nanomaterials:

Firstly, it can combine with ssDNA by π-π stacking non-covalent interactions [21]. Secondly, it is able to quench fluorescence with high efficiency and its water-solubility is better. Thirdly, it prevents ssDNAs who adsorb onto the surface of GO from being digested by enzyme in the biologic environment. Hence, GO, as a superb nanomaterial, would be easily used in biosensors for pathogen detection.

Fluorescence, as a highly sensitive photosignal, has been widely exploited to detect many kinds of materials[22], including tissues, drugs, cells, etc. The fluorescence of FAM could be annihilated by carbon nanomaterials, enabling it is extensively used in detecting *S. paratyphi A*[23,24]. Generally speaking, fluorescence intensity detected by use of one FAM is limited. However, when two FAMs are simultaneously used, the fluorescence would enhanced considerably due to superimposed effect of the fluorescence. Supposing that we employed enzymes to recycle amplification, the signal would be further magnified many times, indicating the detection system has high signal-to-noise ratio and low detection limit.

In the past of decades, many methods have been developed to detect different targets[25]. For example, sandwich system was designed by John to detect *Campylobacter jejuni* based on magnetic bead and quantum dot in 2009[26], and potentiometric analysis was employed by Gustavo to test *Escherichia coli* based on single-walled carbon nanotube in 2010[27]. Although many methods for *S. paratyphi A* detection have been developed, including spectrocopy, PCR, antibody, immunization and gene, they have some limitations[28-30]. The major problems of the spectrocopy methods are weak fluorescence signal and narrow detection range. Furthermore, the use of antibody is expensive, and the procedures of PCR and immunization methods are complicated.

In this study, a very sensitive and specific aptasensor was designed to detect *S. paratyphi A* based on two-FAMs superposition and DNase I-mediated target recycling amplification with weak background signal. Because of fluorescence superposition by two FAM-labelled probes and random digestion of both dsDNA and ssDNA by DNase I, less probes and aptamers are needed. Furthermore, the probes-conjugated aptasensor combined with DNase I hydrolysis provide a lower detection limitation and higher specificity in bacteria rapid detection than previously reported methods. Cosequently, this aptasensor offers a rapid, sensitive and specific way to detect *S. paratyphi A* and has great prospect in other pathogens' determination.

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2. Materials and methods

2.1. Materials and reagents

GO was obtained from Nanjing XFNANO Materials Tech Co. Ltd (Nanjing, China). DNase I was purchased from Thermo. Bacteria were maintained in our laboratory. The stretched aptamer, the probe 1 and probe 2 were synthesized by Sangon Biotechnology CO. LtD (shanghai China). The DNA oligonucleotide sequences were listed below:

Probe 1: 5'-TCTAGA-FAM-3'

Probe 2: 5'-FAM-TCATGA-3'

DA: 5'-TCTAGAGCCACGCGCAGCAATCAAACCCGGCCCCCTGCTCATGA-3'

All these sequences were dissolved with 10mM Tris-HCl buffer (pH 7.5) that was composed of 10mM Tris-HCl (pH 7.5), 2.5mM $MgCl_2$, and 0.1mM $CaCl_2$. Sterile water was used in this assay.

2.2. Apparatus

A LS55 fluorescence spectrophotometer (PerkinElmer, UK) was used to measure fluorescence signals. Both excitation and emission slits were set as 10nm. The excitation wavelength was 480nm and the emission spectra ranged from 300nm to 700nm. The fluorescence intensity at 517nm was applied to estimate the performance of the assay. All measurements were carried out at room temperature. Origin 8.0 software was adopted to deal with the experimental data, and Fluorescence WinLab was used to cope with the fluorescence spectra.

2.3. Preparation of GO

One hundred mL sterile water was added to a conical tube containing 0.1g of GO prepared from natural graphite by the modified Hummers method[31]. The mixture was placed in an ice bath and homogenized ultraphonic by an ultrasonic cell disruptor at 150W for 2h before a homogeneous GO solution was obtained and stored at room temperature for later use.

2.4. Gel-electrophoresis

Gel-electrophoresis was conducted to confirm the feasibility of the method. The product of every reaction was collected. Samples for electrophoresis were prepared individually as aptasensor, aptasensor/GO complex, aptasensor/target complex, mixture of aptasensor/target complex (aptasensor: probe 1 plus DA plus probe 2) and DNase I, mixture of aptasensor 1/target complex (aptasensor 1: probe 1 plus DA) and DNase I, mixture of aptasensor 2/target complex (aptasensor 2: probe 2 plus DA) and DNase I. Each was mixed with 2μL loading buffer and loaded onto 4% agarose gel. The electrophoresis was performed in 10mM Tris-HCl (pH7.5) buffer at 120V for 45min. Then the resulting gel image was photographed under UV light.

2.5. Determination of target by DNase I-mediated fluorescence amplification system

Probe 1, probe 2 and DA were denatured at 95℃ for 5min in water bath and cooled for 10 min in ice bath. Subsequently, 25nM of them were mixed together and incubated at 25℃ for 20min. GO was then added to the mixture and incubated for 10min at 25℃ to quench the fluorescence in the two FAM-modified terminal probes. The fluorescence values were

measured after different concentrations of the bacterium were added into the mixture and incubated at 37℃ for 20min. The amplified fluorescent signals were measured after the addition of 1U DNase I to the solution for 30min at 37℃ in water bath. The experiments to optimize detection conditions were conducted under identical conditions.

2.6. Specificity assay

Specificity assay was conducted by appending different bacteria, including *Salmonella paratyphi A*, *Salmonella. cholerae-suis*, *Escherichia coli K88*, *Staphylococcus aureus* and *Bacillus thuringiensis*, to the reaction system. Bacteria (1×10^{11} cells/mL) were incubated with 25nM aptasensor/GO complex for 20min in 37℃ water bath, and 1U DNase I was subsequently added to the mixtures and incubated for 30min before the fluorescent intensities were measured.

2.7.Samples detection

Milk and water were selected as samples for bacteria detection. Different concentrations of bacteria were added to milk and water in centrifuge tubes, respectively. The aptasensor/GO complex in working solution were mixed with various concentrations of bacteria for 20min and subsequently incubated with 1U DNase I for 30min in 37℃ water bath, and their fluorescent intensities were measured. All experiments were repeated in triplicates.

3. Results and discussion

3.1. Aptamer selection and identification

A group of ssDNA aptamers with high affinity and specificity against *S. paratyphi A* were selected from an enriched oligonucleotide pool by SELEX process. Several other food-borne bacteria were used as counter-selection targets. Through several rounds of positive-SELEX and counter-SELEX, aptamers were sorted, cloned, sequenced, and characterized for binding efficiency. Table 1 showed the aptamer sequences for the bacteria. The aptamer sequences from cell-SELEX were longer than those from protein-SELEX. Taking the cost and high binding affinity into consideration, aptamer 3 was chosen as the experiment sequence.

3.2. Design principle

Recycling amplification was used to detect many targets, such as ATP, bacterium, protein, cell and so on. It was also vital to design a new recycling amplification aptasensor to impove detection signal and decrease detection limit. As shown in Figure 1, DA was a sequence of aptamer 3 flanked by six nucleotides at the 5'-terminal and four nucleotides at the 3'-terminal, which kept a structure similar to that of the initial aptamer 3. The aptasensor, designated as two-FAMs modified aptasensor, was composed of three segments in Figure 2. Segment 1 was the DA, segment 2 was the probe 1 with six bases completely complementary to the 5'-terminal of DA, and segment 3 was the probe 2 with six bases completely complementary to the 3'-terminal of DA. Unlike the traditional quenching agents, GO was widely used because of its high quenching rate, low cost and excellent bio-compatibility. In the absence of target, the aptasensor was hybridized with GO by π-π stacking and the fluorophore of FAM was weak due to GO quenching based on fluorescence resonance energy transfer (FRET).

When target was introduced, however, the aptasensor dissociated from GO due to specific recognition and binding of the aptasensor with the target, resulting in the recovery of fluorescence. Importantly, the fluorescent signal was significantly amplified by DNase I-mediated target recycle process in which all DNA sequences were degraded by DNase I, leading to release of target and FAM fluorophores, and the released target was recycled repeatedly, leading to accumulation of free FAM fluorophores.

To further investigate the feasibility of rapid bacterial detection, fluorescence emission spectra of the aptasensor were analysed under various conditions. As shown in Figure 3, the fluorescence spectrum (f_0) of the aptasensor showed the highest intensity (curve a), whereas more than 97% fluorescence was quenched when appropriate amount of GO was added into the system (curve b), suggesting that aptasensor/GO complex was formed so that GO could quench the FAM fluorescence efficiently. When DNase I was added to aptasensor/GO complex, the fluorescence intensity did not increase obviously (curve c), revealing that the aptasensor was completely inserted in GO and protected from being digested by DNase I. Interestingly, the fluorescence intensities were raised dramatically upon the addition of target (curve d), and elevated to 70% of f_0 upon the addition of target plus DNase I (curve e), demonstrating that the aptasensor was released from GO by its specific binding to target and so the FAM fluorescence was no longer quenched, and that DNase I amplified the fluorescent signal by randomly digesting single- and double-stranded sequences of DNA in the presence of Mg^{2+} and therefore triggering the target recycling process, generating more free FAM fluorophores. Specifically, the fluorescence emission spectra of both aptasensor 1/target

complex and aptasensor 2/target complex were also depicted through the DNase I-mediated target recycling process (curve f and g, respectively). Comparing with the fluorescent intensities of the aptasensor in the absence and presence of DNase I-mediated target recycling (curve d and e, respectively), neither the aptasensor 1/target complex nor the aptasensor 2/target complex showed stronger fluorescence intensity than the aptasensor system. Consequently, the bacteria could be quickly detected through aptamer recognition and DNase I signal amplification.

In order to verify the superiority of the two-FAMs modified aptasensor over the one-FAM modified ones, the fluorescent intensities between one-FAM modified aptasensors (aptasensor 1 and 2) and the aptasensor were compared as well. These data under different conditions were shown in Supplementary Information Figure 1S. The fluorescent recovery rates of aptasensor 1 and 2 were 34% and 27% in the absence of DNase I, 53% and 40% in the event of 1U DNase I, which were 13% - 20% and 17% - 30% lower than those of the aptasensor. Because it has high fluorescence intensities and fluorescent recovery, the aptasensor with high fluorescence intensity and fluorescent recovery exhibited more favorable feasibility.

3.3. Optimization of test conditions

To optimize the detection platform, the concentration and reaction time of GO, the reaction time of target and DNase I were required further experiment. Because the ratio of nanomaterial and biomolecule is a critical factor in the detection system, it is essential to investigate the proper ratio between DA and GO. Nine different GO concentrations (from 0 to

80μg/mL) were prepared and incubated with 25nM aptasensors (25nM aptasensor: 25nM probe 1 plus 25nM DA plus 25nM probe 2; 25nM aptasensor 1: 25nM probe 1 plus 25nM DA; 25nM aptasensor 2: 25nM DA plus 25nM probe 2) in 25℃ water bath for 10min, respectively. Their fluorescence intensities were measured at 517nm with the excitation at 480nm. As displayed in Figure 4A, the fluorescence intensity decreased with an increase in GO concentration. When GO concentration reached 60μg/mL, the fluorescence intensities were quenched by 97% for 25nM aptasensor and by 95% for 25nM aptasensor 1 and 2, indicating that 60μg/mL was the saturation point to quench 25nM aptasensors and thus applied to the subsequent experiments.

To determine the optimal incubation time of GO, target and DNase I, fluorescence intensities were tested every 5min with a reaction solution (25nM aptasensor, 60μg/mL GO, 1×10^{11} cells/mL bacteria, 1U DNase I in Tris-HCl buffer), respectively. When GO was incubated with the aptasensor at 25℃ for 10min, the fluorescence intensity reached the saturation point (Figure 4B). Thus 10min was selected as the optimal incubation time of GO. With the addition of target, the fluorescence intensity increased with time. As it increased quickly in 20min and slowly from 20min to 60min, 20min was chosen for the optimal incubation time of target. Likewise, 30min was supposed as the effective incubation time of DNase I.

3.4. Electrophoresis characterization

To confirm the feasibility of this method, agarose gel electrophoresis was done. As displayed in Figure 5, DNA marker had nine bands (lane 1). The aptasensor presented only one bright

band between 50bp and 25bp after 45min at 60V/cm (lane 2), indicating the aptasensor was established successfully in the reaction buffer. No band was observed with the addition of GO (lane 3), demonstrating that aptasensor was bound to GO, and no free probe was supposed to exist in buffer. Whereas a bright band appeared again after target was added into the buffer (lane 4), suggesting that the aptasensor was pulled off from GO due to its specific binding to target, and the affinity between the aptasensor and target was stronger than π-π stacking. However, no band was displayed when DNase I was added (lane 5), indicating that the aptasensor was completely digested by the enzyme into mono-and oligodeoxyribonucleotides. Similarly, when the samples of DNase I-treated aptasensor 1/target complex and aptasensor 2/target complex were loaded, no bands were seen (lane 6 and lane 7), confirming the successful digestion of aptasensors by DNase I. As a result, our method proved effective and feasible.

3.5. Sensitivity of the aptasensor

In order to explore its sensitivity，which was an important factor for biosensor, parallel assays were performed. Gradient concentrations of target (from 10^3 to 10^{11} cells/mL) were prepared and mixed with the reaction solution which contained aptasensor/GO complex, and the fluorescence emission spectra were recorded. As depicted in Figure 6A, their fluorescence intensities were recovered after the addition of target and exhibited a remarkable increasing tendency from 10^3 to 10^{11} cells/mL. The linear correlation was Y = 44.269x - 100.399, where Y was the fluorescence intensity and X was the concentration of the bacterium in logarithmic phase, respectively (regression coefficient R^2 = 0.99340). The results indicated that the

addition of target led to formation of the aptasensor/target complex and contributed to release of the fluorophores from GO, and the detection sensitivity of this method were improved greatly via fluorescence superposition .

3.6. Signal amplification by DNase I

To achieve signal amplification DNase I was exploited to the system. Ten μL of 10U DNase I was added to 100uL reaction solution and incubated with aptasensor/target complex in 37℃ water bath for 30min. Greater fluorescent signal and lower detection limit (1×10^2 cells/mL) were obtained with the addition of both target and DNase I (Figure 6A, 6B). Moreover, a good linear relation was located from 1×10^2 to 1×10^{11} cells/mL ($Y = 45.186x - 35.824$, $R^2 = 0.99802$). The results displayed that the signal amplification was really achieved by the DNase I-mediated target recycling reaction.

3.7. Specificity of the aptasensor

To evaluate the specificity of the system which was another important factor of aptasensor, parallel assays were done. The controls, including *S. cholerae-suis*, *E. coli K88*, *S. aureus*, *B. thuringiensis*, were incubated with the aptasensor/GO complex and DNase I, respectively. Their fluorescence emission spectra were measured. As shown in Figure 7, the fluorescence intensities of target were much stronger than those of the controls. The results demonstrated that the mehod could distinguish the target from the controls with high specificity. Meanwhile, the aptasensor remained the specificity of aptamer 3 and could identify its target effectively. Therefore, we believe that the aptasensor in our study are highly selective for target and it is a

promising method for the detection of samples with complicated matrix.

3.8. Application for samples

In order to test the application of this system in real samples, we determined some bacteria in milk and water. As observed in Figure 8, their fluorescence intensities were between the aptasensor and aptasensor/GO complex when various bacteria in samples were detected by this method. However, the fluorescence emission spectra of non-target bacteria detection were lower than those of target. The observed results demonstrated well that the established method is competent in real samples detection.

Conclusions

In this study, we have successfully developed a simple, highly selective and sensitive aptasensor for *Salmonella paratyphi A* detection by using GO as an efficient fluorescence quencher and DNase I as a competent signal amplifier. As it can detect target with a wide linear range (from 1×10^2 to 1×10^{11} cells/mL) and a low detection limit (1×10^2 cells/mL), the aptasensor is superior to other methods (10^6 cells/mL detected by spectrophotometry and 10^5 cells/mL by fluorescence). More importantly, its operational process is simpler than them, that require extraction of specific genes (iroB and gyrA gene) or use complex multi-enzymes (exonuclease I and exonuclease III). GO employed in this system has its intrinsic advantages over other materials, such as low cost, high quenching efficiency, homogeneous dispersibility and so on. In addition, significant fluorescence signals can be attained with high sensitivity and specificity when this aptasensor is adopted to test target in samples. DNase I was first

used for bacteria detection based on its unique properties of digesting dsDNA and ssDNA randomly and simultaneously, resulting in degradation of aptasensor from fluorophore-aptasensor/target complex and release of target and fluorophore. Also, the target recycling makes it possible to release more fluorophores and hence to achieve an amplified fluorescenct signal. Furthermore, the two-fluorophore aptasensor produces higher fluorescence intensity than the one-fluorophore aptasensor in this system. Meanwhile, its quantity is small and fluorescence intensity upbeat. Consequently, the aptasensor should be considered as a new tool for other sample detections since it has high sensitivity and specificity.

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Footnotes

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[2]Abbreviations usage: DA, designed aptamer; FRET, fluorescence resonance energy transfer; GO, graphene oxide; DNase I, deoxyribonuclease I; FAM, carboxyfluorescein; SELEX, systematic evolution of ligands by exponential enrichment; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; *S. paratyphi A*, *Salmonella paratyphi A* (isolated from patients infected with *Salmonella*); *S. aureus*, *Staphylococcus aureus* (ATCC25923); *S. cholerae-suis*, *Salmonella cholerae-suis* (ATCC10708); *B. thuringiensis*, *Bacillus thuringiensis* (CCTCC200016); *E. coli K88*, *Escherichia coli K88* (CVCC216); PCR, Polymerase Chain Reaction; AFLP, Amplified Fragment Length Polymorphism. ATP, adenosine triphosphate.

Table 1.

Selected Types	Aptamer Agents	Sequence (from 5' to 3')	Kd[nM]
Cell-SELEX	Apt 10	GATGATGGACGTATATCGTCTCCCATGAATTCAGTCGGACAGCG	73±9
	Apt 22	ATGGACGAATATCGTCTCCCAGTGAATTCAGTCGGACAGCG	47±3
	Apt 45	ATGGACGAATATCGTCTCCCAGTGAATTCAGTCGGACAGC	68±6
	Apt 60	CGCCCACCCATAATGGATCAGGGCGGGCACCACGATG	56±9
Protein-SELEX	Apt 1	CGAAGGGGCTATGCCGCCTACATAGACCGTCACGA	49±6
	Apt 2	GGCCGGCAATACGGCCGAGCCCGGGGTTCCTCCGA	61±7
	Apt 3	GCCACGCGCAGCAATCAAACCCGGCCCCCTGCTCC	27±5
	Apt 4	TGGCCAGAGTACGAGTAAGGGAGGTCACAACCTTA	65±9

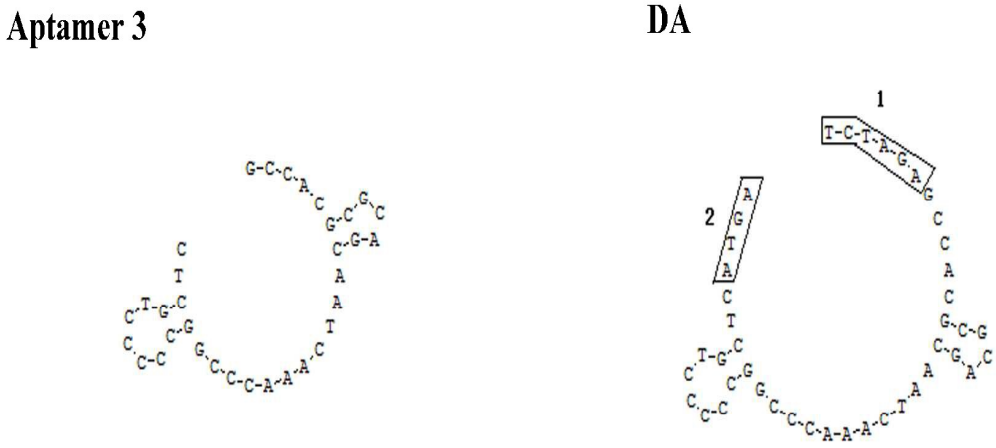


Figure 1.

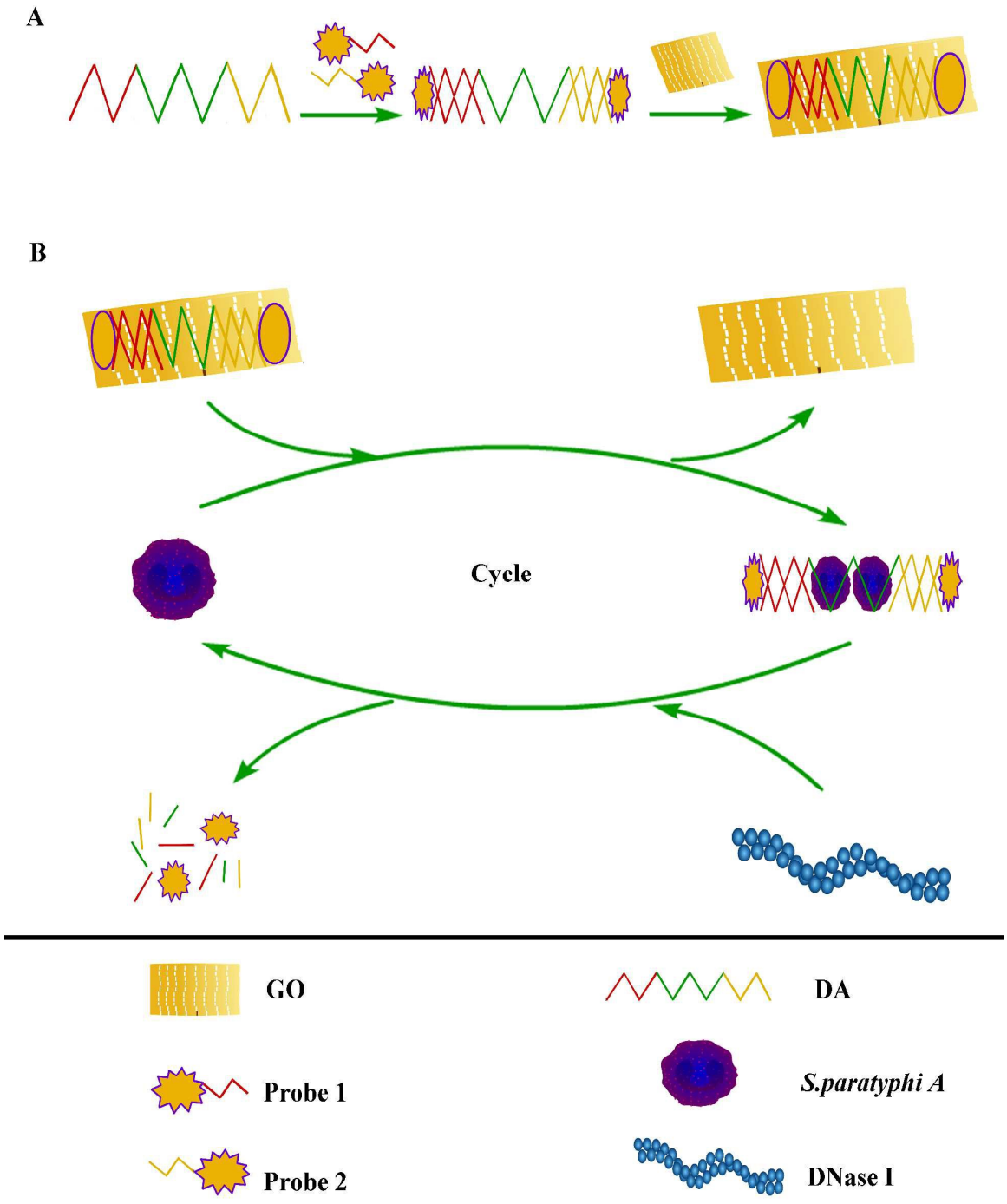


Figure 2.

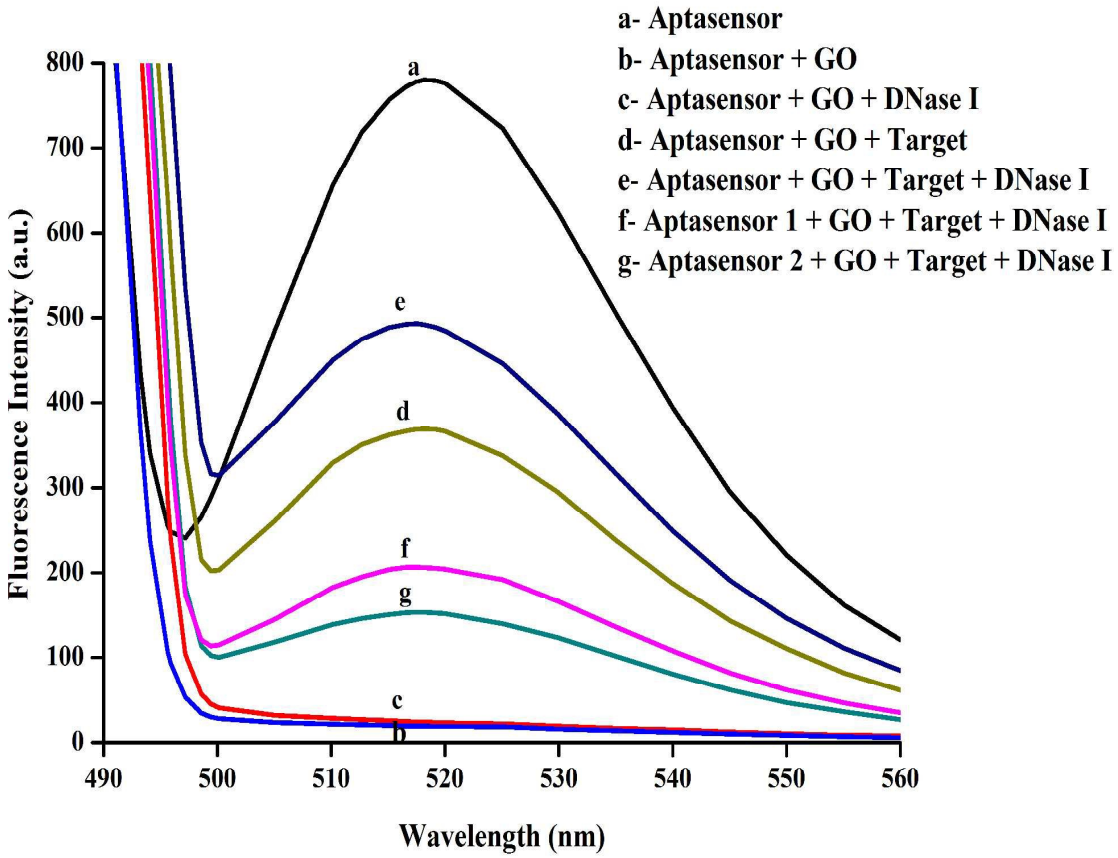


Figure 3.

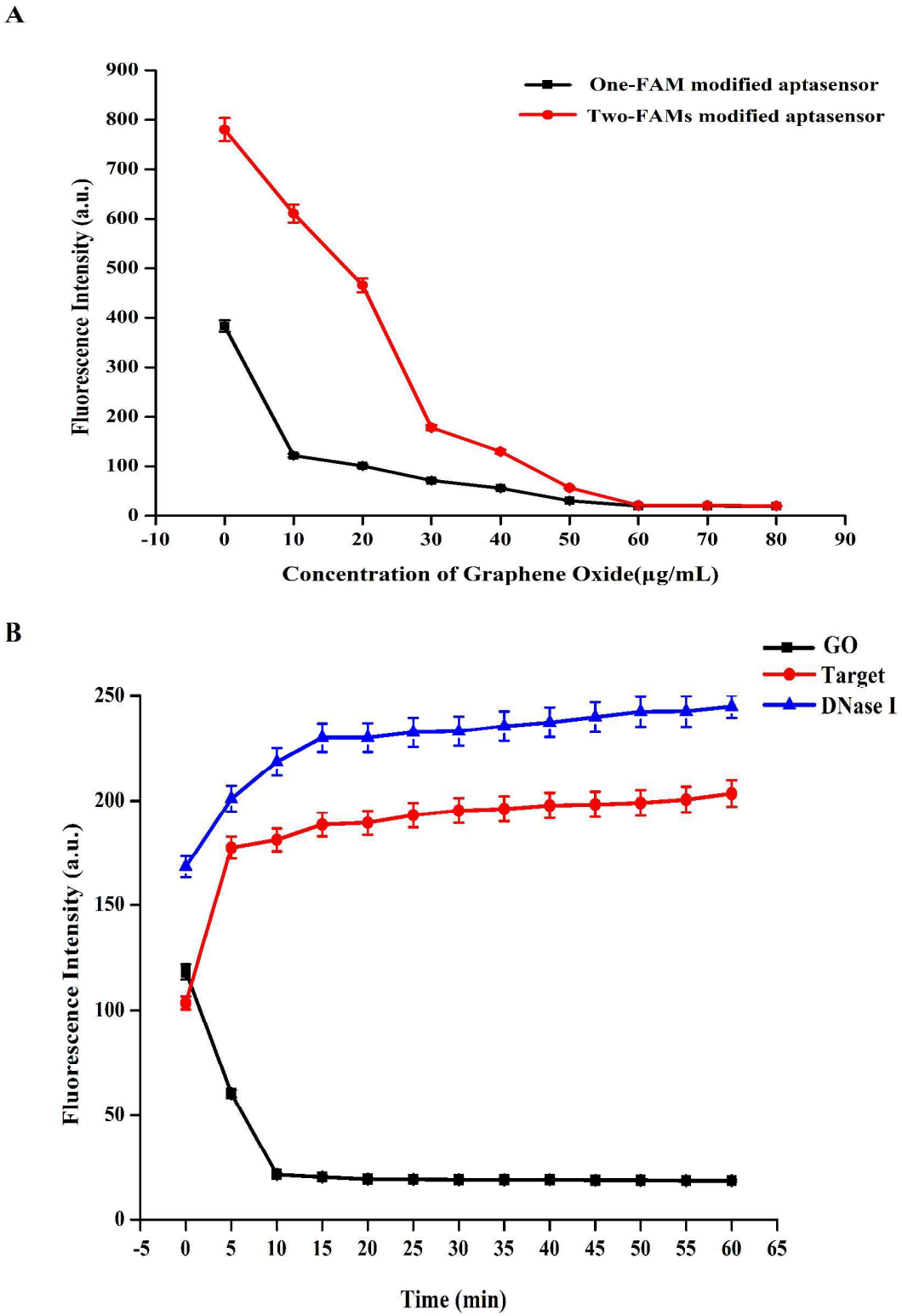


Figure 4.

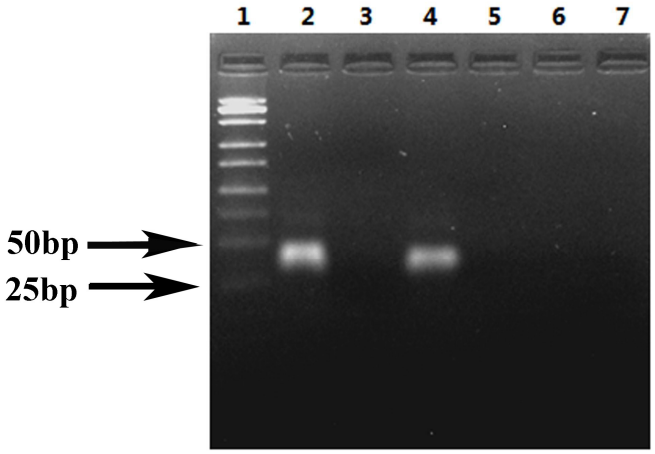
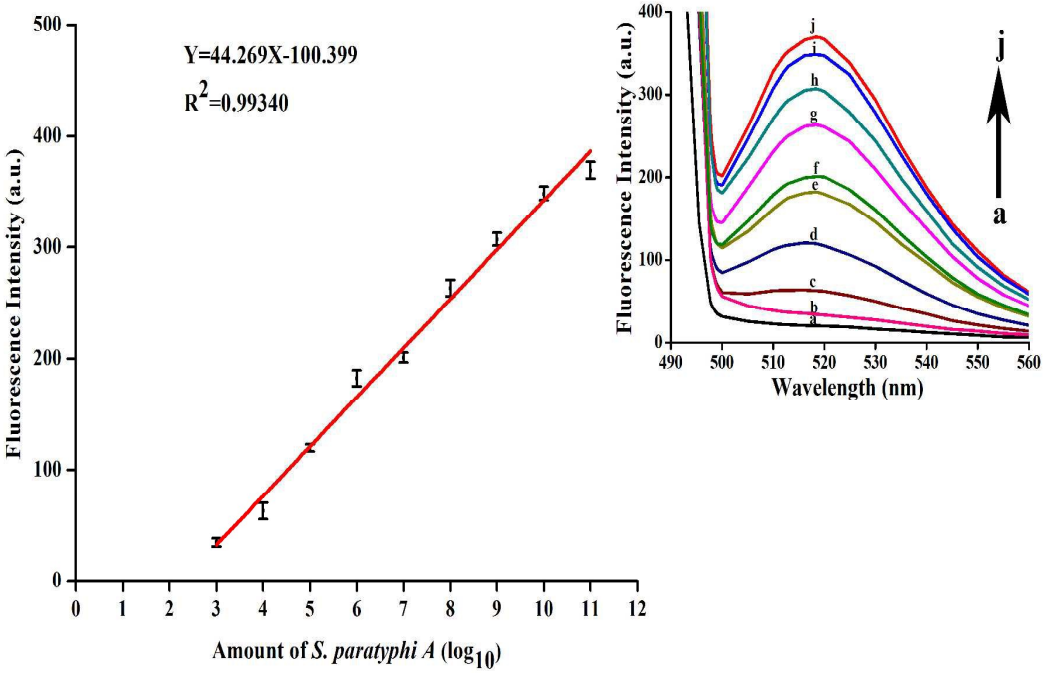


Figure 5.

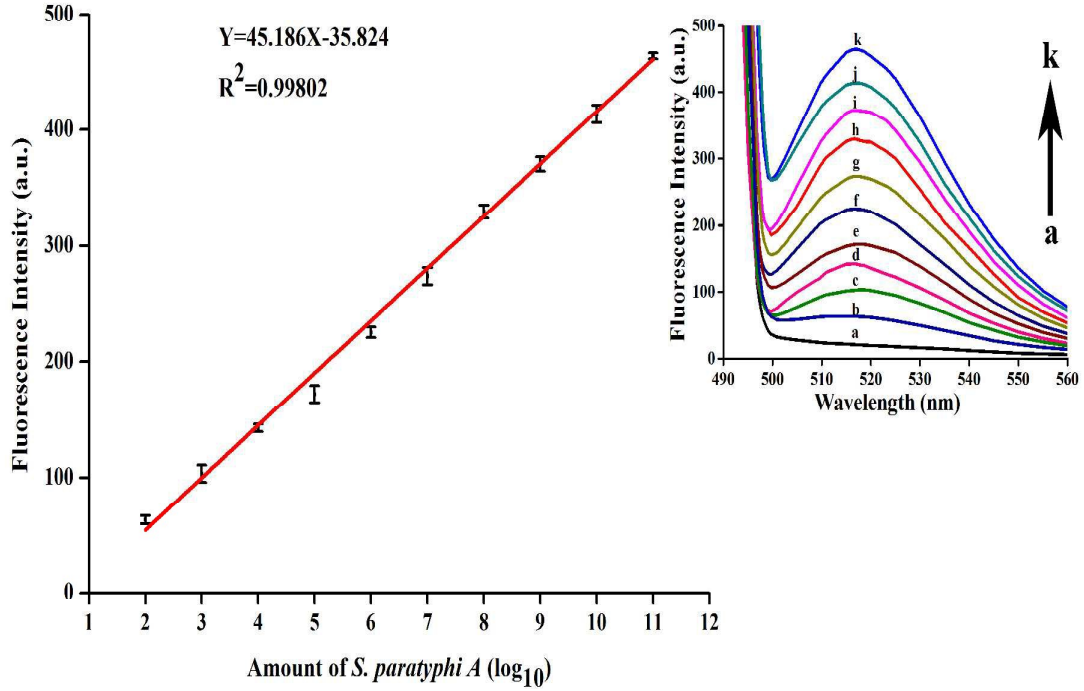
503

A



504

B



505

506 **Figure 6.**

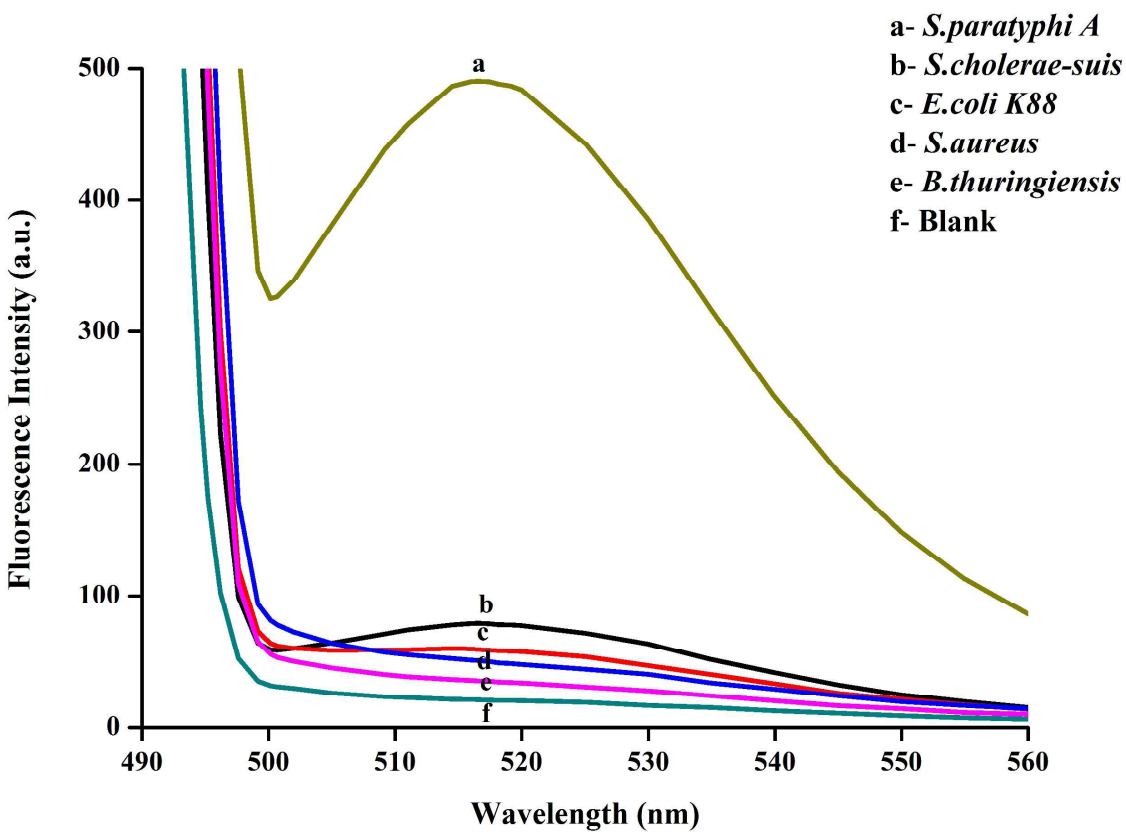


Figure 7.

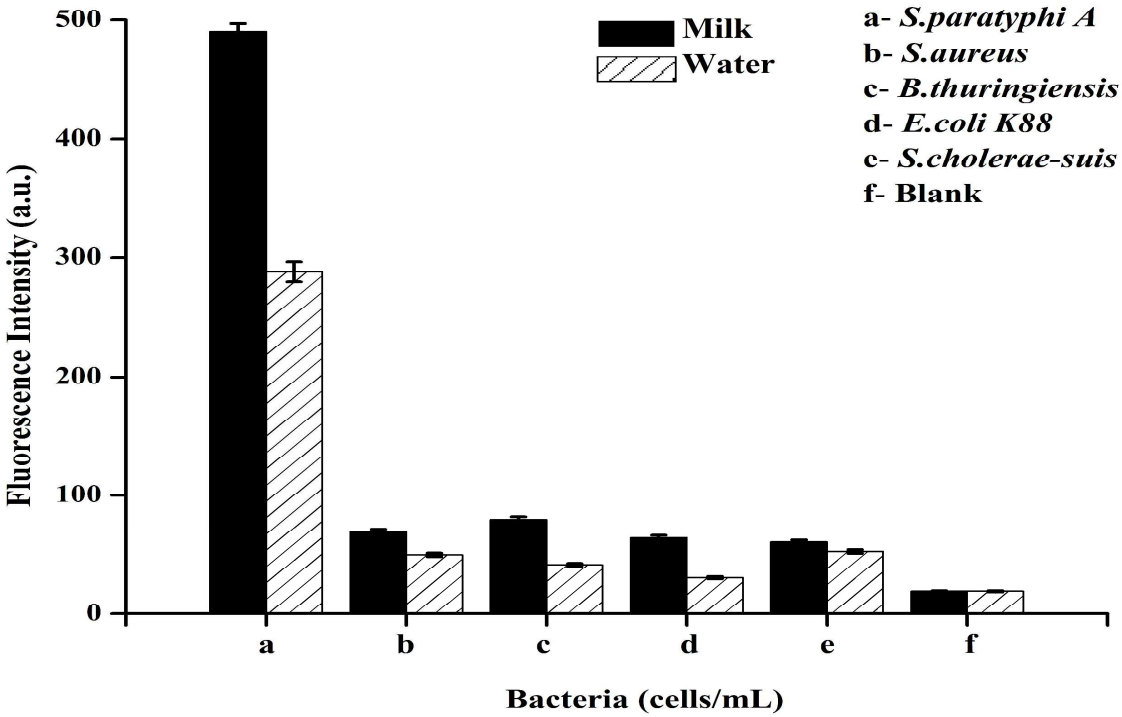


Figure 8.

Captions for figures

Table 1. Eight aptamers of *S.paratyphi A* from two types of SELEX.

Figure 1. Predicted secondary structures of the selected aptamer 3 and DA using DNAMAN software. Their minimum free energy are -2.69 kilocalorie/mol. Part 1 stands for the outstretched sequence of the aptamer 3 at 5'-end and Part 2 stands for the outstretched sequence of aptamer 3 at 3'-end in DA.

Figure 2. Schematic presentation of the detection system via GO-dependent nanoquencher and DNase I-mediated target cyclic amplification. (A) Constructions of the aptasensor and quenching effect of GO. Probe 1 and 2 combine with the DA's terminals by base complementary pairing rule accordingly. Adding GO (60μg/mL) to the reaction solution lead to quenching of fluorescence. (B) Target contributes to the recovered fluorescence and DNase I do to the amplified fluorescence further.

Figure 3. The feasibility of the proposed aptasensor. Fluorescence intensities obtained from the "product" at different stages: the initial fluorescence intensity of the aptasensor (curve a); aptasensor/GO complex (curve b); aptasensor/GO complex + DNase I (curve c); aptasensor/ GO complex + target (curve d); aptasensor/GO complex + target + DNase I (curve e); aptasensor 1/GO complex + target + DNase I (curve f); aptasensor 2/GO complex + target + DNase I (curve g). The aptasensor components: 25nM DA, 25nM probe 1, 25nM probe 2. The aptasensor 1 components: 25nM DA, 25nM probe 1. The aptasensor 2 components: 25nM DA, 25nM probe 2. GO concentration: 60μg/mL. Target concentration: 1×10^{11} cells/mL. DNase I concentration: 1U. λ_{em} = 517nm and λ_{ex} = 480nm.

Figure 4. (A) The fluorescence quenching effect of GO at different concentrations. The one-FAM modified aptasensors' components: 25nM DA, 25nM probe 1 or 25nM probe 2. The two-FAMs aptasensor components: 25nM DA, 25nM probe 1 and 25nM probe 2. (B) Optimization of the reaction time for aptasensor/GO complex (solid square),

aptasensor/target complex (solid diamond), aptasensor/target complex + DNase I (solid triangle). The fluorescence spectra measured at 5min time intervals in 10mM Tris-HCl (pH7.5) buffer. GO concentration: 60μg/mL. Target concentration: 1×10^7 cells/mL. DNase I concentration: 1U. Error bars indicate standard deviation (n = 3).

Figure 5. Native polyacrylamide gel electrophoresis analysis of the "product" at different stages: the DNA ladder (lane 1); the aptasensor (lane 2); aptasensor/GO complex (lane 3); aptasensor/GO complex + target (lane 4); aptasensor/GO complex + target + DNase I (lane 5); aptasensor 1/GO complex + target + DNase I (lane 6); aptasensor 2/GO complex + target + DNase I (lane 7).

Figure 6. (A) The target detection at different concentrations without DNase I. The fluorescence intensities depend on the *S. paratyphi A* concentrations ranging from 10^3 to 10^{11} cells/mL. Fluorescence intensities in target detection: fluorescence spectral response of aptasensor/GO complex (curve a); fluorescence emission spectra after aptasensor/GO complex incubated with different concentrations of target (curves b to j correspond to the concentrations ranging from 10^3 to 10^{11} cells/mL). (B) The target detection at different concentrations with DNase I. Fluorescence intensities depend on the *S. paratyphi A* concentrations ranging from 10^2 to 10^{11} cells/mL. Fluorescence intensities in target detection: fluorescence spectral response of aptasensor/GO complex (curve a); the fluorescence emission spectra after aptasensor/GO complex incubated with different concentrations of target and 1U DNase I (curves b to k correspond to the concentrations ranging from 10^2 to 10^{11} cells/mL). The aptasensor components: 25nM DA, 25nM probe 1, 25nM probe 2. GO concentration: 60μg/mL. DNase I concentration: 1U. λ_{em} = 517nm and λ_{ex} = 480nm. Error bars indicate standard deviation (n = 3).

Figure 7. The detection specificity of the proposed aptasensor. The concentrations of target and other nonspecific targets were 1×10^{11} cells/mL, respectively. The aptasensor components:

25nM DA, 25nM probe 1, 25nM probe 2. GO concentration: 60μg/mL. DNase I concentration: 1U. The fluorescence intensities were read at λ_{em} = 517nm and λ_{ex} = 480nm. Error bars indicate standard deviation (n = 3).

Figure 8. Ability verification of the approach to detect samples. Fluorescence emission spectra of different bacteria at the 1×10^{11} cells/mL in milk (solid) and the 1×10^{7} cells/mL in water (diagonal).

Highly Sensitive Fluorescent Aptasensor for *Salmonella paratyphi A* Via DNase I-mediated Cyclic Signal Amplification

Xing Yan, Wenkai Li, Keyi Liu and Le Deng

Graphical Abstract:

An elegant aptasensor was developed for dual fluorimetric determination of *Salmonella paratyphi A* through DNase I-assisted target recycling enlargement.