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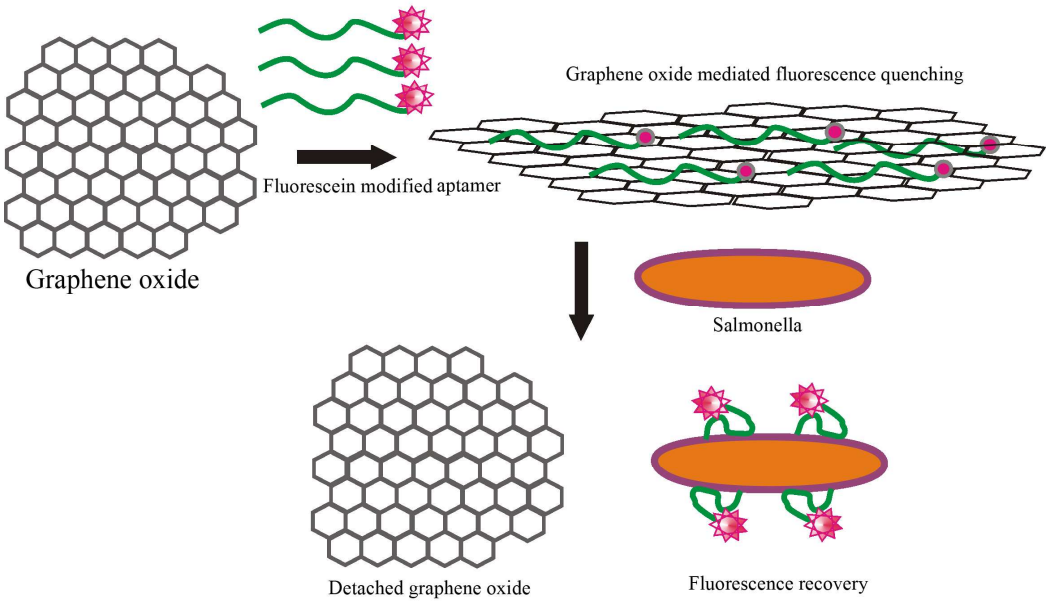

In this *communication*, we report a rapid and cost-efficient assay for the detection of S.*enteritidis*. Specific aptamer with fluorescence labeled of S.*enteritidis* is absorbed into the graphene oxide, and fluorescence is quenched owing to fluorescence quenching ability of graphene oxide. In the presence of S.*enteritidis*, the aptamer would release from the graphene oxide to obtain a significant fluorescence recovery. This aptasensor can detect as low as 40 CFU/ml of S.*enteritidis* in 30 min, and can fulfill the demand of multiplex detection. The cost-effective, rapid and simple aptasensor offers a promising method for food-borne pathogens monitoring during food processing.

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ARTICLE TYPE

A simple aptamer biosensor for Salmonellae *enteritidis* based on fluorescence -switch signaling graphene oxide

Wei Wu‡ [a], Zhiyuan Fang ‡ [a,c], Shiming Zhao[a,b], Xuewen Lu[a], Luxin Yu[a], Ting Mei[a], Lingwen Zeng[a,*]

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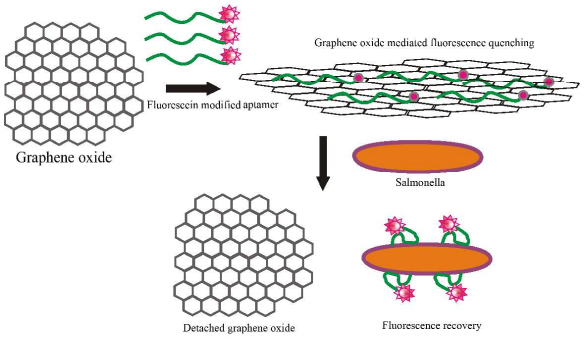
In this *communication*, we report a rapid and cost-efficient assay for the detection of S.*enteritidis*. Specific aptamer with fluorescence labeled of S.*enteritidis* is absorbed into the graphene oxide, and fluorescence is quenched owing to fluorescence quenching ability of graphene oxide. In the presence of S.*enteritidis*, the aptamer would release from the graphene oxide to obtain a significant fluorescence recovery. This aptasensor can detect as low as 40 CFU/ml of S.*enteritidis* in 30 min, and can fulfill the demand of multiplex detection. The cost-effective, rapid and simple aptasensor offers a promising method for food-borne pathogens monitoring during food processing.

Food-borne pathogen contamination is one of the major concerns in food industries. The prevention and control of food-borne pathogen contamination have attracted worldwide attentions. Failure to detect the food-borne pathogens may lead to a dreadful effect. However, pathogenic microorganisms are prominent contaminants in food and drinking water [1]. Salmonellae is considered to be one of the most dangerous pathogens to human and a leading cause of food-borne disease. S. *enteritidis* is the most common serotype of salmonellae in food poisoning in the last two decades. The infectious dose of S. *enteritidis* lies between 1 to 10 colony-forming units (CFU) [2]. Therefore, the routing monitoring of S. *enteritidis* is very important. Commonly used methods for S. *enteritidis* detection rely on conventional culture-based tests. These methods are costly, laborious, time-consuming, and are unable to meet the requirement of real-time detection [3]. Therefore, new methods based on antibody immunoassay and polymerase chain reaction (PCR) have been developed [4, 5]. They have high sensitivity and selectivity, but tedious procedures and highly trained personnel are still required. In addition, PCR based methods can only tell the presence of target nucleic acids in the sample, false-positive results may be encountered when cells have broken down [6]. Hence, it is of high importance to have a fast, reliable, sensitive and cost-effective method for S. *enteritidis* detection.

The nanomaterial graphene oxide (GO) is a two-dimensional single carbon atomic layer arranged in a honeycomb lattice [7]. Recently, the GO with distinct chemical properties has expanded its territory beyond electronic and chemical applications toward bio-analytical areas, such as the graphene mediated fluorescence quenching based biosensors [8, 9]. Like other nanomaterials, such as gold nanoparticles (AuNPs) and quantum dots (QDs), GO-based nanoprobes have been successfully used for the fluorimetric detection of nucleic acids, proteins, metal ions and intracellular cell imaging [10-13]. It is a promising material for pathogen detections in food and environment, owing to its excellent fluorescence quenching ability [14].

Aptamers are single stranded oligonucleotides that can naturally fold into three-dimensional structures, therefore have the capability to bind biotargets with high specificity [15]. GO nanosheet has a high affinity for single strand DNA (ssDNA), and the interaction is reversible. Thus, based on the fluorescence resonance energy transfer (FRET) property, the fluorescein modified aptamer/GO nanocomplex can be an ideal tool for real-time bacteria detection. In this short communication, we report a reliable and rapid approach for viable S. *enteritidis* detection using a fluorescein modified S. *enteritidis* aptamer. S. *enteritidis* aptamer (S-aptamer) was synthesized chemically with fluorophore carboxyfluorescein FAM modification according to a previous study [16]. Fluorescence is quenched when the FAM modified aptamer (FAM-aptamer) is absorbed onto the GO nanosheet. In the presence of S. *enteritidis*, the S-aptamer can detach from the GO nanosheet and bind to S. *enteritidis*, forming an aptamer/S. *enteritidis* duplex. Hence, significant fluorescence recovery can be observed immediately (Scheme 1).



Scheme 1 Schematic illustration of the aptsensor for Salmonella detection.

S. *enteritidis* at 10^6 CFU/mL was first used for the preliminary tested. Briefly, FAM modified S-aptamer was incubated with GO to form aptamer-FAM/GO nanocomplex. After 5 min of

incubation, almost 90% fluorescence quenching was observed. Then, S. *enteritidis* suspension at 10^6 CFU/mL was added. As expected, S-aptamer binds to S. *enteritidis* and forms a stable duplex. Consequently, the detaching of S-aptamer from the surface of GO nanosheet results an extraordinary fluorescence recovery (Fig.S1, ESI†).

The selectivity of this aptamer-based biosensor (aptasensor) was tested using three different strains of pathogens, S. *Paratyphi,* S. *Cholerae-suis*, and *E. coli* K88. Significant fluorescence recovery was observed when 10^6 CFU/mL of S. *enteritidis* was incubated with aptamer-FAM/GO (Fig.S1, ESI†), indicating the successful aptamer delivery and sensing of S. *enteritidis*. As negative controls, S. *Paratyphi,* S. *Cholerae-suis*, and *E. coli* K88 were also incubated with S-aptamer-FAM/GO at the same concentration, but no detectable fluorescence change was recorded (Fig. S1, ESI†).

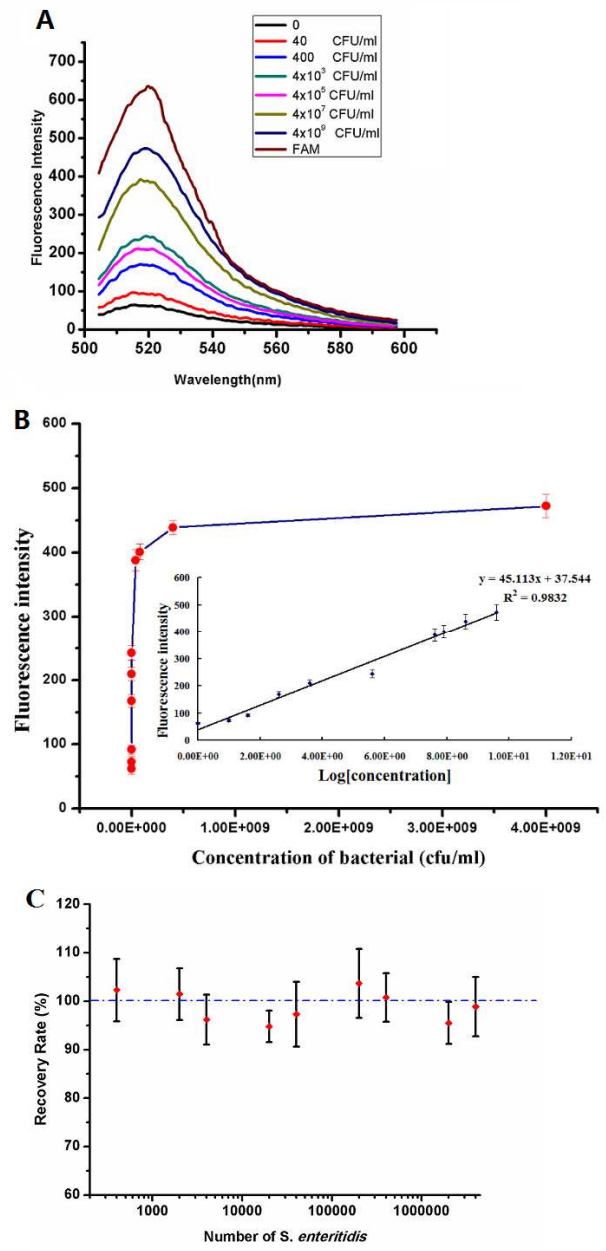


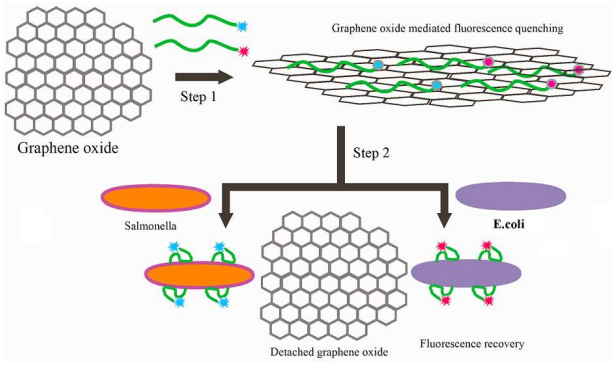
Fig. 1. Performance of the GO-based aptasensor for S. *enteritidis* detection. A: Sensitivity of the aptasensor. From bottom to top, the concentrations of S. *enteritidis* are 40 CFU/mL to 4×10^9 CFU/mL; B: Calibration curve of the aptasensor with different amounts of S. *enteritidis*. The fluorescence intensity at 520 nm was mean value of three measurements. Error bars indicate standard deviations from three independent experiments. C: Distribution of the recovery rate of S. *enteritidis* spiked milk detection. Error bars indicate standard deviations from three independent experiments.

To further confirm the selectivity of this aptasensor, S-aptamer without FAM-modification was used for the competitive binding of S. *enteritidis*. Dramatic fluorescence decreasing was observed at the presence of unmodified S-aptamer (Fig.S1, ESI†). The results show that this aptasensor has high selectivity for S. *enteritidis*. It has no cross-reactivity to other Salmonella serovars or other pathogens.

The efficiency of fluorescence quenching has great effect on the background and the sensitivity of the assay. Thus, different concentrations of GO, ranging from 0 to 50 μg/mL, were incubated with 200 μM of aptamer-FAM in 500 μL of PBS to get the best working concentrations of GO and aptamer. When the concentration of GO was 10 μg/mL, more than 50% of the fluorescence was quenched. As the concentration increased to 20 μg/mL or higher, 84%-90% of the fluorescence was quenched (Fig.S2A, ESI†). No increment of quenching efficiency was observed when more than 20 μg/mL of GO was used. Therefore, 20 μg/mL was chosen as the optimizing GO working concentration. The recovery of fluorescence intensity was also affected by the incubation time. The optimum amount of time for incubation was tested using 10^6 CFU/mL of S. *enteritidis*. The fluorescence intensity reached the plateau after 20 min of incubation. Herein, 20 min was chosen as the optimum incubation time (Fig. S2B, ESI†).

After optimizing the experimental parameters, we explored the sensitivity of this aptasensor. The limit of detection was calculated by three standard deviations from the mean of blanks, which was the fluorescence intensity responding to 0 CFU/ml of S. *enteritidis*. The fluorescence intensity for 0, 40, 400 CFU/ml of S. *enteritidis* detection were 61.82, 91.92, 167.71, respectively. The limit of detection (LOD) of this aptasensor is the S. *enteritidis* concentration responding to the fluorescence intensity of the mean blank value plus 3 standard deviations (SD). From 20 groups of blank, the mean blank value was 61.82 and SD was 9.63. The responding fluorescence intensity of LOD was 90.17. The fluorescence intensity of 40 CFU/ml of S. *enteritidis* was 91.92, which was greater than 90.17. Therefore, the LOD of our aptasensor was 40 CFU/ml. Therefore, the sensitivity was calculated to be 40 CFU/mL. To further improve the sensitivity, a pre-enrichment is needed, such as aptamer modified magnetic beads [17]. Besides, novel fluorescent dye, such as quantum dots QDs, can be used for the substitution of traditional dyes. The QDs have higher quantum yield, which will increase the sensitivity significantly [18, 19]. Fig. 1A shows the spectra of this aptasensor responding to a broad range (from 40

to 4×10^9 CFU/mL) of S. *enteritidis*. Fig. 1B presents the corresponding fluorescence intensity responses of the aptasensor when loaded with various amounts of S. *enteritidis*. From the figure, the linear range of our aptasensor was from 40 cfu/ml to 4×10^9 cfu/ml, with an equation of *y=45.113x+37.544* (x refers to the log[concentration]).



Scheme 2. Schematic of principles for the multiplex detection.

Skim milk (3%, w/v) spiked with different concentrations of S. *enteritidis* was used to test the performance of this aptasensor in real sample detection. The final concentrations of S. *enteritidis* were 4×10^2, 2×10^3, 4×10^3, 2×10^4, 4×10^4, 2×10^5, 4×10^5, 2×10^6, 4×10^6 CFU/mL. All the S. *enteritidis* spiked samples were detected as positive results. The results show that the presence of biological matrix such as milk has no influence on the performance of this aptasensor. Moreover, the assay has a good recovery rate ranging from 93% to 104% (Fig. 1C), which can be explained by the high affinity between S-aptamer and S. *enteritidis*. These results show that this aptasensor is applicable for the pathogen detection in real samples.

As food products may be contaminated by one or more types of pathogens simultaneously, multiplex detection is needed in food-safety monitoring. Hence, E. *coli* and S. *enteritidis* at 10^7 CFU/mL were tested in this study. Scheme 2 shows the principle of aptasensor in the multiplex detection. Cy3 modified E. *coli* aptamer (E-aptamer) [20] and FAM modified S-aptamer were used in the detection Obvious fluorescence recovery, up to 90%, was observed after the adding of these two pathogens (Fig. 2A,B).

Herein, the FRET between GO and fluorescin in aptamer was applied in the real-time detection of bacteria in this study. Non-covalent and reversible binding between GO and aptamers enables fast and precise delivery of aptamers. The quenching ability of GO in a broad range of spectrum enables the use of variety of fluorescent dyes, rendering this aptasensor with capability of multiplex detection. In conclusion, the advantages of this aptasensor such as rapidity (~30 min), low-cost, simplicity, sensitivity and multiplexing ability, make it a promising candidate for the use in pathogen detection.

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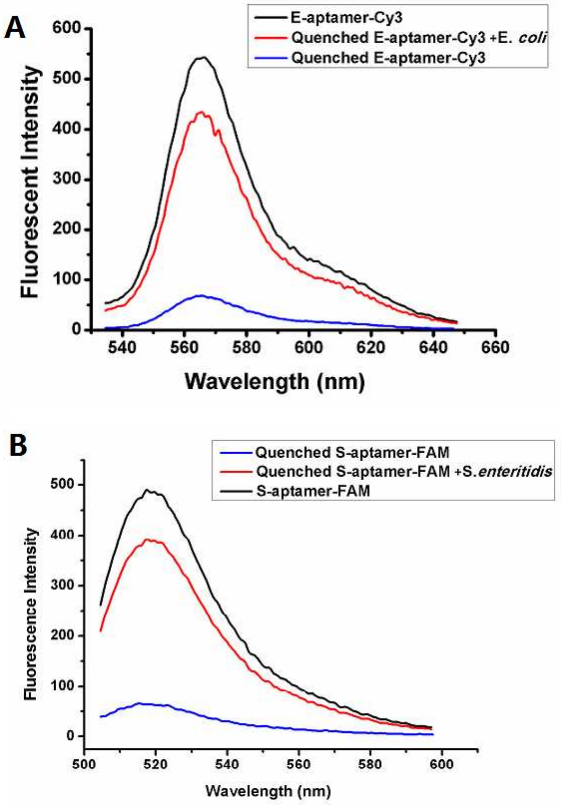

Fig. 2. Aptasensor for the simultaneous detection of S. *enteritidis* and *E. coli*. Fluorescence spectra of aptasensor responding to the simultaneously addition of *E. coli O157(A) and* S. *enteritidis (B)*.

Notes and references

[a] *Key Laboratory of Regenerative Biology, South China Institute for Stem Cell Biology and Regenerative Medicine, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510530, China.*

[b] *College of Life Science and Technology, Anhui University, Hefei, China*

[c] *Affiliated Tumor Hospital of Guangzhou Medical University, Guangzhou 510095, China.*

* *Corresponding author. Tel.: +862032015245.*

E-mail: zeng_lingwen@gibh.ac.cn

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‡ These two authors contribute equally to this work.

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