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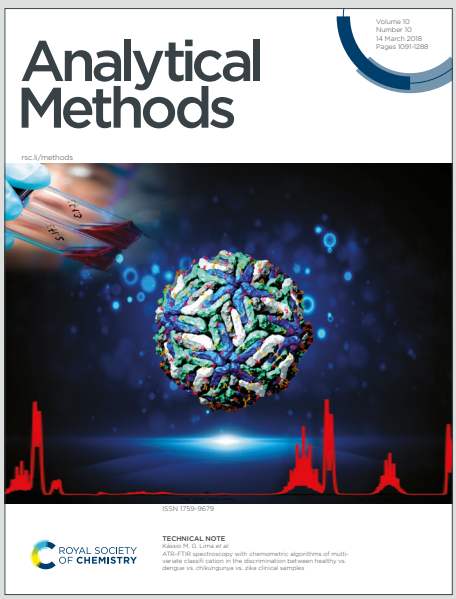

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Fluorescence biosensor for *Salmonella typhimurium* detection in food based on nano-self-assembly of alendronic acid modified upconversion and gold nanoparticles

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Abstract

A simple and quick responsive fluorescent biosensor for *Salmonella typhimurium* detection based on the recognition of aptamer coupled with alendronic acid (ADA)@upconversion nanoparticles (UCNPs) and gold nanoparticle (AuNPs) has been developed. Briefly, aptamer can adsorb on the surface of AuNPs by "Au-S" bond to protect AuNPs from aggregation in high concentrated salt solution. Then AuNPs and UCNPs linked by the electrostatic adsorption, which leads to decrease in fluorescence peak at 541 nm based on fluorescence resonance energy transfer (FRET) between UCNPs and AuNPs. In the presence of *Salmonella typhimurium*, the "Au-S" bond was broken, and the fluorescence intensity in 541 nm recovered. Under the optimal condition, the correlation between the concentration of *S. typhimurium* and the intensity of the fluorescent biosensor signals were observed to be linear within the range of 1.16×10^2 - 1.16×10^7 CFU/mL (R^2 = 0.9912), and the detection limit of the developed biosensor was observed to be at 36 CFU/mL. Furthermore, the proposed method was successfully applied to detect *Salmonella typhimurium* pathogen in food samples with satisfactory results.

Keywords: Fluorescence biosensor; ADA@UCNPs; AuNPs; *Salmonella typhimurium*

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1. Introduction

Salmonella typhimurium, as one of the most common food pathogen, can select human as host and cause diseases through intestinal infection.[1] In past several decades, almost 100 million of food-borne illnesses was caused by *Salmonella typhimurium* and it directly caused more than $365 million economic costs.[2] In addition, The World Health Organization (WHO) reported that Salmonellosis is the most frequent food-borne illness.[3] Therefore, the development of a rapid, specific and sensitive method to detect *Salmonella typhimurium* at early stage is an urgent need to ensure food safety.

The traditional culture-based approach as the gold standard method is still most reliable to detect food-borne pathogens with high accuracy and sensitivity at present, but this method cannot meet the requirement of rapid screening detection due to long detection time (4-6 d). In recent years, many new detection assays such as polymerase chain reaction (PCR) and enzyme linked immunosorbent assay (ELISA) for food-borne pathogens have been reported instead of the conventional time-consuming culture methods.[4-7] Among these new methods, the PCR skill for food-borne pathogens detection has progressed to a great extent. And the skill of multiplex PCR,[8] multiplex real-time PCR and Loop-mediated isothermal amplification

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(LAMP) were developed based on the traditional PCR for detection of food-borne pathogens.[9,10] There are many advantages including rapid, relatively accurate and simultaneous multiplex pathogens detection by using PCR-based method. Nonetheless, the requirement of professional operator and expensive equipment are obstacles and difficult to overcome at present. ELISA is recognized as a cheap, rapid and convenient food-borne pathogens detection method. However, the sensitivity of traditional ELISA is one of colorimetric methods and it is not enough to detect the polluted-pathogen samples. Therefore, it is critical to establish a new, cheap, accurate and rapid method to detect food-borne pathogens.

As an alternative food-borne pathogens detection method, many electrochemical biosensors and fluorescent biosensors are developed.[11,12] A similar technology that use aptamer or antibody has been utilized to identify food-borne pathogens in the above mentioned biosensors. Therefore, these biosensors have the advantages of high sensitivity and selectivity.[13] However, almost all electrochemical biosensors applied in food sample is easy to be interference, and the relative limit of detection (LOD) in food sample is higher than LOD in pure water. For instance, Li et al. developed an electrochemical biosensor based on quartz crystal Au electrode with LOD of 10^2 CFU/mL in buffer and 10^3 CFU/mL in food sample.[14] Nowadays, fluorometric assays are most popular analytical method due to their unique

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properties such as simple operation and high efficiency,[15,16] and have been extensively used in development of biosensors to detect food-borne pathogens. Among various fluorescent biosensors, the fluorescent biosensors based on upconversion nanoparticle possess several advantages over the biosensors consists of organic fluorophores or quantum dots.[17-19] First, the upconversion fluorescence biosensors have stronger anti-interference ability own to their optical properties including low auto-fluorescence background,[20,21] long luminescence lifetimes and high anti-Stoke shift. Second, the upconversion nanoparticles have low toxic and can be functionalized easily. These optical properties together with multifunctional states simple modifications imply that UCNPs have good potential for food-borne pathogens detection applications. Therefore, it is highly desirable to develop a sensitive and convenient upconversion fluorescence biosensor for detection of *Salmonella typhimurium*.

To make an upconversion fluorescence biosensor, it is very important to select a fluorescence acceptor. Recently, many colormetric sensors consist of gold nanoparticles (AuNPs) have been reported, and these sensors are highly recognized due to unique properties of AuNPs including size,[22,23] chemical surface, shape, high extinction coefficients, and compatibility with surface functionalization.[24] Some fluorescence biosensors consist of gold nanoparticles as fluorescence acceptor and fluorescence material as

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fluorescence donor for detection of bacterial were reported.[24,25] For instance, Pebdeni et al. reported a fluorescence turn-on aptasensor consist of green carbon quantum dot and gold nanoparticles to detect staphyloccus aureus in milk and orange juice with LOD of 10 CFU/mL in 1h;[26] Srinivasan et al. developed label-free aptasensors consist of Rhodamine B and gold nanoparticles for detection of Salmonella typhimurium with LOD of 464 CFU/mL.[27] Those above gold nanoparticles-based fluorescence biosensors finish target bacteria with high sensitivity. However, those fluorescence material applied in biosensors inevitably interfered fluorescence background, which can affect the accuracy of the detection. Therefore, considering UCNPs and gold nanoparticles to develop a fluorescence biosensor for detection of Salmonella typhimurium is necessary.

Aptamer, a single-stranded nucleic acid (RNA or DNA) can bind a wide range of targets with high affinity, high sensitivity. Many research works about targets-specific DNA aptamers selection was reported.[28,29] The selectivity can be enhanced by using whole-cell SELEX (systematic evolution of ligands by exponential enrichment). Nowadays, lots of different aptamer-based biosensors including colorimetric aptasensors,[30] fluorescence-based aptasensors and SERS aptasensor for detection of food-borne pathogens have been reported.[31,32] For instance, Feng et al. developed a gold nanoparticle-based colorimetric aptasensor for detection of

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Shigella flexneri with a wide working range of 10^2-10^6 CFU/mL in 20 min;[33] Duan et al. reported a SERS-based aptasensor approach based on Au@Ag core/shell nanoparticles to detect *Salmonella typhimurium* in food with a low limitation of 15 CFU/mL in 30 min;[34] Liu et al. made a fluorescence-based aptasensors based on FRET between CdSe/ZnS quantum dots and gold nanoparticles for detection of *vibrio parahaemolyticus* in 30 min with a low limitation of 10 CFU/mL without pre-enrichmen.[35] It can be found that these above aptamer-based biosensors have good sensitivity and finish detection work in short time due to aptamer's properties. Therefore, aptarmer for binding *Salmonella typhimurium* can be considered to build an aptamer-based upconversion fluorescence biosensor.

In the present work, we aim to develop nano-self-assembly fluorescent biosensor consists of upconversion nanoparticles, aptamer and gold nanoparticles for detection of *Salmonella typhimurium*. Without addition of *Salmonella typhimurium*, aptamer were adsorbed to the surface of gold nanoparticles, leading to upconversion nanoparticles-gold nanoparticles composite due to the electrostatic adsorption, resulted in the quenched upconversion fluorescence by the FRET between upconversion nanoparticle and gold nanopartice. With addition of *Salmonella typhimurium*, aptamer cannot be adsorbed to the surface of gold nanoparticles, leading to separation of upconversion nanoparticles and gold nanoparticle,

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consequently recovering the upconversion fluorescence. Owing to the high sensitivity of aptamer and upconversion fluorescence, the limit of detection of the developed method reached to 36 CFU/mL. Meanwhile, the detection performances of the developed method was evaluated on the basis of specificity, anti-interference, precision, and practicability by using other common foodborne pathogens and *Salmonella typhimurium* spiked pork, chicken and milk samples. The results demonstrated that the proposed fluorescence biosensor showed great potential for sensitive detection of *Salmonella typhimurium* in food samples.

2. Materials and methods

2.1 Materials

Rare-earth chloride hexahydrate: $ErCl_3 \cdot 6H_2O$ (99%), $YCl_3 \cdot 6H_2O$ (99%), $YbCl_3 \cdot 6H_2O$ (99%), $GdCl_3 \cdot 6H_2O$ (99%), oleic acid (90%) and 1-octadecene (90%) were purchased from sigma-Aldrich company (Shanghai, China). Sodium hydroxide (NaOH), ammonium fluoride (NH_4F), phosphate buffer solution (PBS), hydrogen tetrachloroaurate hydrate ($HAuCl_4 \cdot 3H_2O$), alendronic acid (ADA), Sodium chloride (NaCl) and other reagent were purchased from Alfa Aesar (Shanghai, China). The sequence of *Salmonella typhimurium* aptamer: 5'-HS-C_6-TA T GGC GGC GTC ACC CGA CGG GGA CTT GAC ATT ATG ACA G-3' was synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China). *Salmonella typhimurium* (ATCC

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14028) was purchased from BeNa Culture Collection (www.bncc.org.cn). *E.coli* (ATCC 35150), *Vibrio parahemolyticus* (ATCC 17802), *Staphylococcus aureus*(CICC 26074), *Shigella flexneri* (CMCC 51571), *Listeria monocytogenes* (CICC 21622) and *Vibrio cholerae* (CICC21615) were supplied by Enzyme Engineering Laboratory, Nanjing Agricultural University.

2.2Instrumentation

UV-Vis spectra was recorded with UV-1800 (Jindao, Japan) using a 1 cm path length quartz cell. Transmission electron microscopy (TEM) image were obtained using a Tecnai G2 F30 transmission electron microscopy. Power X-ray diffraction (P-XRD) spectra was measured with a Siemens D5005 instrument (Bruker AXS, Ltd., Germany) equipped with Cu Kα radiation from 5° to 70° at a scan rate of 2°/min. The zeta potentials were measured on Malvern Zetasizer Nano (Malvern Instruments Ltd, U.K). Fourier transform infrared spectroscopy (FT-IR) was performed with a Nicolet IR200 Fourier transform infrared spectrophotometer (Nicolet Co., U.S.A.).

2.3 Bacteria species

The *E.coli*, *Vibrio parahemolyticus*, *Staphylococcus aureus, Shigella flexneri, Listeria monocytogenes* and *Vibrio cholerae* were used as controls

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for diagnostic specificity of the developed biosensor to detect *Salmonella typhimurium*.

2.4 Bacterial strain culturing

All the bacteria were grown in sterile Luria-Bertani (LB) media in an incubator-shaker at 37℃ over night. The bacteria concentration was determined by plate counting and also by measuring the optical density (OD_{600nm} = 0.3) using a 1 cm optical path length quartz curvette, while a serial dilution was performed to achieve a range of bacterial culture concentrations. The bacteria pure culture was collected by centrifugation at 8000 rpm for 3 min at room temperature and then washed three times and re-suspended in PBS.

2.5 Synthesis and surface modification of $NaYF_4$:Yb/Er UCNPs

Thermal decomposition procedure was applied to synthesize oleic acid-capped $NaYF_4$:Yb/Er UCNPs according to the previous literature with some modifications.[36] Briefly, a round-bottom flask with three neck containing 2 ml of $RECl_3$ (0.2 M, RE = Y (48%), Gd (30%) Yb (20%), Er (2%)), 3 ml of oleic acid and 7 mL of 1-octadecene was heated at 160 ºC for 30 min with vigorous stirring. Then 10 ml of methanol solution containing 0.148 g NH_4F and 0.1 g NaOH was slowly added into the flask and stirred at 50 ºC for 30 minutes. Thereafter, the mixture was heated and degassed at 70 ºC to remove methanol for 40 min. Afterwards, the mixture was heated at

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300 ºC and maintained for 1 h under N_2 protection. Finally, the product was washed with cyclohexane-ethanol (1:2) two times and dried under vacuum at 60 ºC overnight .

Water-soluble upconversion nanoparticles were prepared with the modification of ADA using the following procedures:[37] 100 mg of UCNPs, 25 mg of ADA, 4mL of $CHCl_3$, 2 mL of ethanol and 3 mL of water dispersed by ultrasound for 5 minutes in 50 mL centrifuge tube. Then 3-4 drops of HCl (1 mol/L) was added to the above mixture and kept for 1 h under continuous stirring. Finally, the ADA-UCNPs solution was washed by water three times and dried in a vacuum oven at 50 ºC overnight.

2.6 Synthesis of AuNPs

AuNPs were fabricated according to the previous literature with appropriate modifications.[38] All glassware was cleaned by the mixture solution (HCl/HNO_3 = 3:1) and used for AuNPs synthesis. 5.0 ml of $HAuCl_4$ (1.0% wt) and 95 ml of deionized water were added into 250 ml flask. Next, the mixture was heated to 100 ºC under vigorous stirring, and 10 ml of trisodium citrate solution (1.0% wt) was added rapidly into the above hot solution. Then, the mixture was boiled for 10 min. Finally the yielded AuNPs were filtered with 0.45 μm filter membrane and stored at 4 ºC.

2.7 Selectivity

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The specificity of the designed fluorescence biosensor for *Salmonella typhimurium* detection was evaluated by the target of different types of food-borne pathogens. In the present work, 10^4 CFU/mL *Salmonella typhimurium* and 10^6 CFU/mL other food-borne pathogens were used to explore the selectivity of the developed biosensors.

2.8 Fluorescence detection of *Salmonella typhimurium*

All fluorescence detections were performed under room temperature. Various concentrations of *Salmonella typhimurium* solution were prepared from the stock solution of *Salmonella typhimurium* using serial dilution to determine the sensitivity limits of the biosensor. First, 30 μL of 1.16×10^1 -1.16×10^8 CFU/mL *Salmonella typhimurium* were mixed with 10 μL of 0.3215 μmol/mL aptamer solution. After 5 min incubation, 300 μL of AuNPs and 50 μL of 0.003 mg/mL UCNPs were added to the mixed solution for 7 min. Then 10 μL of 0.5 moL/mL NaCl was mixed into the above solution for 9 min. Finally, the solution of developed biosensor containing different concentrations of *Salmonella typhimurium* was determined using the fluorescence spectrometer system for measuring the fluorescence intensity at 541 nm.

2.9 Application in real samples

Chicken, milk and pork samples were purchased from the local supermarkets. First, chicken and pork samples were homogenized in a

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blender, and all three samples were artificially contaminated with 10^3-10^4 CFU/mL of *Salmonella typhimurium* culture media.[39] Then, the mixture was centrifuged at 12000 rpm for 10 min at 4℃ and washed with PBS three times. All three samples were re-suspended in 10 mL of PBS solution and quantitatively analyzed with our developed biosensor and flat colony counting method.

3 Results

3.1Characterization

The shape, size, crytsallinity and surface modification of the UCNPs were evaluated using TEM, P-XRD, FT-IR. As be seen in Fig. 1A, the TEM image of UCNPs shows uniform sized shaped particles (50 nm) and good dispersibility. Fig. 1C shows the XRD profile of $Na(Y_{0.57}Yb_{0.39}Er_{0.04})F_4$. From the image high intensity planes at (100), (001), (110), (201),(300), (211), low intensity planes at (200), (111), (100), (210), (002), (102), (112), (220), (202), (310) were indicating dominant hexagonal phase $Na(Y_{0.57}Yb_{0.39}Er_{0.04})F_4$ (JCPDS file No. 28-1192) in the crystal structure. The surface properties of ADA modification before and after upconversion nanoparticles were determined by FT-IR. In Fig. 1D, the FT-IR spectra shows vibration modes with sharp bond at 1468 cm^{-1}, 1577 cm^{-1}, 2862 cm^{-1} and 2933 cm^{-1} which were assigned to the symmetric and asymmetric stretching vibration modes of carboxylic group (COO^-) and the methylene

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(-CH_2-), respectively (Fig. 1D (a)), and the characteristic peaks at 3473 cm^{-1} was assigned to the stretching vibration of hydroxide radicals (-OH) of oleic acid on the surface of UCNPs. When oleic acid-capped upconversion nanoparticles were modified by ADA, one new well-defined characteristics peaks emerged at 1135 cm^{-1} followed by the disappearance of the peaks at 1468 cm^{-1} and 1557 cm^{-1}. Gold nanoparticles were also characterized by TEM. As shown in Fig. 1B, the TEM image shows fairly uniform with a size of about 15 nm.

3.2 Proof principle

The principle for detecting *Salmonella typhimurium* based on FRET between ADA modified UCNPs and AuNPs is schematically described in Fig. 2. First, AuNPs were insured to self-aggregate by NaCl solution, leading to visible color change from red to blue-gray. After addition of sulfhydryl-functionalized aptamer with AuNPs, the aptamer-AuNPs complexes informed through “Au-S” chemistry, resulting redispersation of AuNPs. With addition of UCNPs, aptamer-AuNPs can be absorbed to the surface of UCNPs through the effect of electrostatic adsorption. In the absence of *Salmonella typhimurium*, The fluorescence intensity at 541 nm of biosensors decreased due to the FRET pair of UCNPs and AuNPs. In the presence of *Salmonella typhimurium*, aptamer can recognize the target of *Salmonella typhimurium* and formed aptamer-*Salmonella typhimurium*

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complexes, leading re-aggregation of AuNPs, resulted in the quenched fluorescence restoration through removing the effect of FRET between UCNPs and AuNPs. To confirm the fluorescence quenching mechanism, the absorption spectra, fluorescence spectra and zeta potentials were measured (Fig. 3). The zeta potentials of UCNPs and aptamer-AuNPs are shown in Fig. 3A, ADA modified UCNPs were positively-charge with zeta potential value of +45.7mV, and the zeta potential of aptamer-AuNPs was -65.6mV. This enabled the distance between UCNPs and aptamer-AuNPs less than 10 nm via electrostatic force. In addition, the characterization of fluorescence spectra of UCNPs and the absorption of AuNPs is shown in Fig. 3B, and the FRET occurred due to spectral overlap between absorption spectrum of AuNPs and fluorescence spectrum of UCNPs in 541 nm when the distance of UCNPs and AuNPs is less than 10 nm.

3.3 Optimization of assay conditions

To obtain better sensing performance for *Salmonella typhimurium* detection, the concentration ratio of UCNPs and GNPs was optimized. The detail of upconversion fluorescnece spectra (inset in Fig. 4A) and fluorsscence intensity at 541 nm study of 150-350 μL GNPs with 50 μL of 0.003 mg/mL UCNPs is shown in Fig. 4A. The fluorescence intensity at 541 nm decreased with GNPs. When the volume of GNPs reached to 300 μL, the fluorescence intensity tended to be steady. Thus 300 μL GNPs was selected

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to be the best optimal condition. The absorption spectra of GNPs-Nacl solution with different reaction time is shown in Fig. 4B. The absorption spectrum tended to be steady after 9 min, which comfirms the reaction between GNPs and Nacl is completed. Thus 9 min was used as optimal time betwween GNPs and Nacl. The fluorescnece spectra of UCNPs (inset in Fig. 4C) and fluorescence intensity at 541 nm with different concentration of Nacl is shown in Fig. 4C. The fluorescence intensity increasing with Nacl and tended to be steady when the concentration of Nacl reached to 0.5 moL/mL. Thus 0.5 moL/mL Nacl was selected as the optimal concentration. The absorption spectra of GNPs-Nacl and mixed aptamer solution with different reation time is shown in Fig. 4D. The absorption peak at 550 nm tended to be steady after 7 min. Therefore, 7 min was used to be the optimal time. Finally, the fluorescnece spectra (inset in Fig. 4E) of UCNPs-GNPs-Nacl solution and fluorescence intensity at 541 nm mixed with different aptamer concentarion is shown in Fig. 4E. It was indicated that the fluorescence intensity at 541 nm was decreasing gradually unitil with addition 0.3125 μM then it was stable. Thus, 0.3125 μM was selected as the optimal concentration of aptamer.

3.4 Detection specificity

To evaluate the analytical selectivity and specificity of this proposed biosensor, 10^4 CFU/mL of *Salmonella typhimurium* and 10^6 CFU/mL other

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food-borne pathogens as non-tagert were tested using the developed method. As shown in Fig. 5, the upconversion fluorescnece intensity is significantly higher at 541 nm in the presence of *Salmonella typhimurium* by comparing to other food-borne pathogens. The high specificity can be ascribed to the high selectivity of the aptamer to *Salmonella typhimurium*, which is purified by high performance liquid chromatograph. Besides, the selectivity of our developed biosensor was compared with other research work (Table S1). These results demonstrate that developed biosensor has good selectivity against *Salmonella typhimurium*.

3.5 Analytical performance of the proposed biosensors towards *Salmonella typhimurium* determination

Under the above-mentioned optimum condition, 1.16×10^{1}-1.16×10^{8} CFU/mL *Salmonella typhimurium* suspension was tested respectively and the corresponding fluorescence spectra were recorded. As shown in Fig. 6A, upon an increase in the concentration of *Salmonella typhimurium,* the fluorescence intensity at 541 nm increased gradually. Thus, the value of fluorescence intensity at 541 nm was proportional to the concentration of *Salmonella typhimurium*. Based on this phenomenon, the fluorescence intensity at 541 nm was used to analysis various concentration of *Salmonella typhimurium* quantitatively (Fig. 6B). The LOD (36 CFU/mL) can be calculated by $3S_b/S$ (S_b represents the standard deviation of 10 blank

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fluorescence measurements and S is the slope of calibration plot). The upconversion fluorescence intensity at 541 nm was found to be linearly related with the concentration of *Salmonella typhimurium* ranging from 1.16×10^2 to 1.16×10^7 CFU/mL, and the linear regression equation is $y=115.35x +505.19$ ($R^2= 0.9912$). The developed biosensor was compared with some previously reported methods, and the results were summarized in table 1. It was confirmed that the developed biosensor had high sensitivity in the detection.

3.6 Detection of *Salmonella typhimurium* in food samples

To verify the applicability of the developed biosensor, we detected simulated sample spiked with *Salmonella typhimurium* before and after incubation for 2, 4 h at 37 ℃. The recoveries (89%-103%) of this biosensor for detecting food samples are shown in table 2, where food samples detected by the developed method and flat colony counting method showed a similar result after incubation for 2 h and 4 h, indicating that the biosensor satisfied the requirements for quantitative detection of *Salmonella typhimurium*. Therefore, this biosensor is operative for detecting *Salmonella typhimurium* in food samples.

4 Conclusion

In conclusion, a novel and sensitive fluorescent biosensor was developed for sensing *Salmonella typhimurium* in food sample based on

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FRET between ADA modified UCNPs and AuNPs. *Salmonella typhimurium* were specific recognized by aptamer, causing the "Au-S" bond broken, resulting the fluorescence at 541 nm increasing. The change in the fluorescence intensity at 541 nm response was used to quantify the concentration of *Salmonella typhimurium* in the linear dynamic range of 1.16×10^2 - 1.16×10^7 CFU/mL with an LOD of 36 CFU/mL. Besides, this biosensor also exhibited good specificity and anti-interference ability by comparing with other bacterial species. Finally, our developed biosensor can be used to detect *Salmonella typhimurium* in pork, chicken and milk in a simple and rapid way.

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Compliance with Ethical Standards

Ethical Approval

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

Informed Consent

Not applicable

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Table caption

Table 1 Comparision of the method with other methods applied for the determination of *Salmonella typhimurium*

Table 2 Sample detection for different concentration of *Salmonella typhimurium* by our developed biosensor and flat colony counting method

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Figure legends

Fig. 1 TEM image of alendronic acid modification upconversion nanoparticle (UCNPs) (A) and gold nanoparticle (AuNPs) (B). XRD patterns of UCNPs (C). FT-IR spectra of upconversion before (a) and after (b) alendronic acid modification upconversion (D).

Fig. 2 Schematic presentation of fluorescent nanoprobe based on fluorescence resonance energy transfer (FRET) between upconversion nanoparticle and gold nanoparticles for detection of *Salmonella typhimurium*.

Fig. 3 (A) The zeta-potential of alendronic acid modification upconversion nanoparticle and aptamer-gold nanoparticles (Apt-AuNPs). (B) The fluorescence spectra of UCNPs and the absorption spectra of AuNPs .

Fig. 4 (A) upconversion fluorescence intensity (UCF) towards different volume of AuNPs (B) Optimization of reaction time after addtion of Nacl (C) UCF upon addition different concentration of Nacl (D) Optimization of hybridization time after addition of aptamer (E) UCF toward different concentration of aptamer.

Fig. 5 Upconversion fluorescence (UCF) intensity at 541 nm of the developed biosensor in the presence of (a) *Salmonella typhimurium*, (b) blank, (c) *E.coli*, (d)*Vibrio parahemolyticu*, (e) *Staphylococcus aureus*, (f) *Shigella flexneri*, (g)*Listeria monocytogenes*, (h)*Vibrio cholerae.*

Fig. 6 (A) Upconversion fluorescence spectra of our developed biosensor in the presence of different concentrations of *Salmonella typhimurium* (1.16×10^{1}-1.16×10^{8} CFU/mL). (B) Calibration plot obtained using the upconversion fluorescence intensity at various concentrations of *Salmonella typhimurium*.

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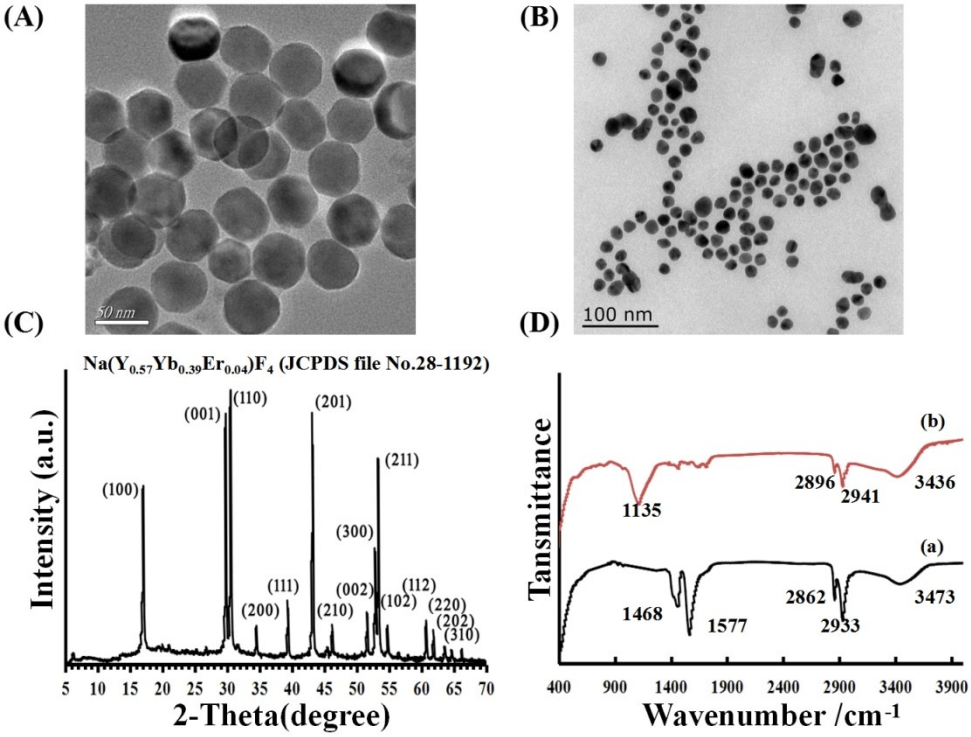

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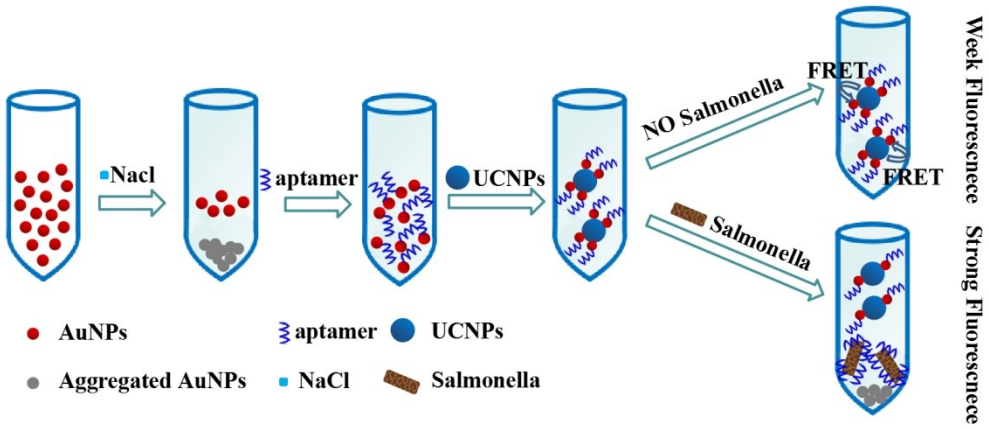

Scheme.1 Schematic presentation of fluorescent nanoprobe for detection of Salmonella typhimurium

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Fig. 2 Schematic presentation of fluorescent nanoprobe based on fluorescence resonance energy transfer (FRET) between upconversion nanoparticle and gold nanoparticles for detection of Salmonella typhimurium

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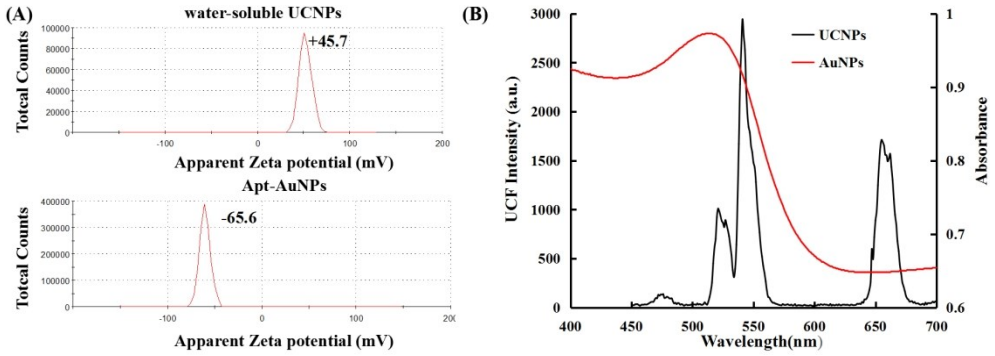

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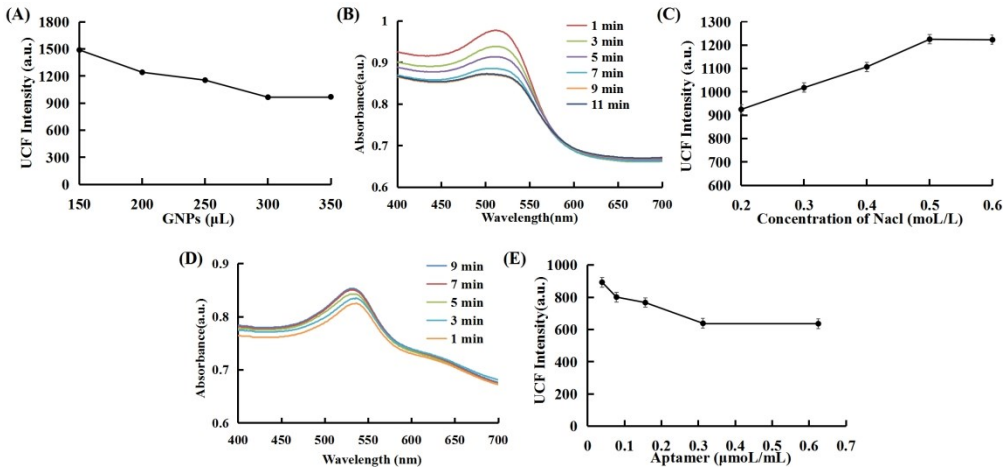

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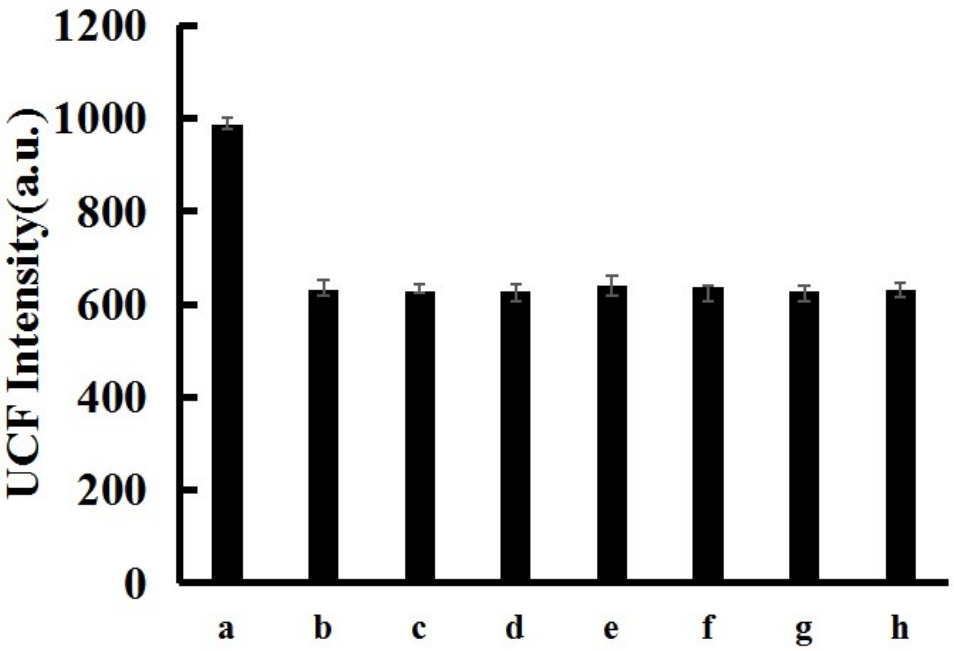

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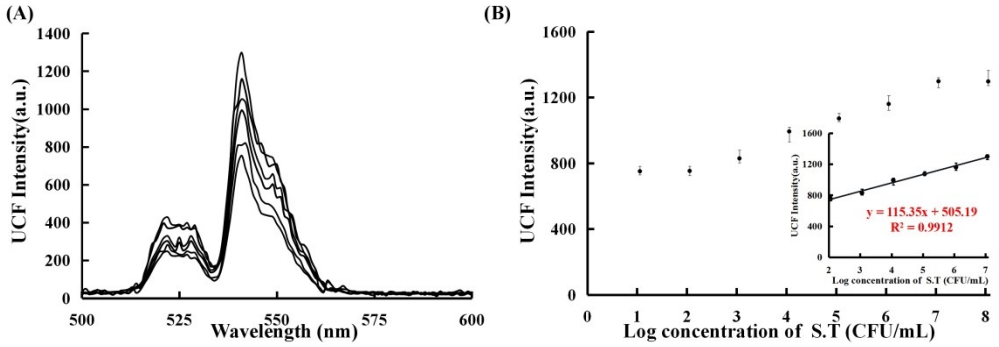

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Table 1: Comparision of the method with other methods applied for the determination of *S.typhimuriumn*

Methods	Linear range (CFU/ml)	LOD (CFU/ml)	Detection time	Reference
Fluorescent probe	10^2-10^5	30	1.5 h	12
SERS-based aptasensor	15-1.5×10^6	15	< 2h	30
QCM-based aptamer	7.9×10^2-7.9×10^6	100	< 1h	1
Our developed method	1.16×10^2 - 1.16×10^7	36	0.5 h	

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Table 2: Sample detection for different concentration of S.typhimurium by our developed biosensor and flat colony counting method

sample	Spiked levels (CFU/mL)	our developed method (Mean[a]±SD[b])(CFU/mL)	flat colony counting method (Mean[a]±SD[b]) (CFU/mL)	Recovery (%)
water	10^3	$(0.914\pm0.031)\times10^3$	$(0.953\pm0.022)\times10^4$	91
	10^4	$(1.032\pm0.023)\times10^4$	$(0.982\pm0.016)\times10^5$	103
chicken	10^3	$(0.892\pm0.017)\times10^3$	$(0.931\pm0.025)\times10^4$	89
	10^4	$(0.931\pm0.010)\times10^4$	$(0.957\pm0.032)\times10^5$	93
pork	10^3	$(0.972\pm0.033)\times10^3$	$(0.931\pm0.009)\times10^4$	97
	10^4	$(0.922\pm0.019)\times10^4$	$(0.963\pm0.027)\times10^5$	92
milk	10^3	$(1.012\pm0.011)\times10^3$	$(1.094\pm0.017)\times10^4$	101
	10^4	$(0.941\pm0.021)\times10^4$	$(0.912\pm0.012)\times10^5$	94

[a]Mean concentration;[b] Standard deviation