

Signal-off colorimetric and signal-on fluorometric dual-mode aptasensor for ultrasensitive detection of *Salmonella Typhimurium* using graphitic carbon nitride

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ABSTRACT

Food safety is severely burdened by the prevalence of foodborne pathogens and the diseases they cause, necessitating the development of rapid, easy-to-use, highly sensitive, and reliable detection methods. Here, a signal-off colorimetric and signal-on fluorometric dual-mode detection method for *Salmonella Typhimurium* (*S. typhimurium*) was developed based on its unique interaction with aptamer DNA and graphitic carbon nitride (GCN). In the absence of a target *Salmonella* species, 6-carboxyfluorescein (FAM)-labeled aptamers are adsorbed on the surface of GCN primarily via a π - π interaction, resulting in reduced fluorescence of FAM through GCN-mediated quenching as well as improved peroxidase-like activity of GCN to generate intense blue color through facilitated electrostatic attraction between the negatively charged aptamer and positively charged 3,3',5,5'-tetramethylbenzidine (TMB) substrate. The introduction of *S. typhimurium* to the sample solution causes the detachment of the aptamer from GCN due to its higher affinity for *S. typhimurium* than GCN, thereby rapidly reducing the colorimetric signal and recovering the fluorescence. We successfully determined the number of *S. typhimurium* using this method in a remarkably short duration (10–30 min), highlighting its rapidity. The limit of detection values for *S. typhimurium* were as low as 8 and 3 CFU/mL when using colorimetric and fluorometric methods, respectively. Moreover, this method can be used to detect *S. typhimurium* spiked in real vegetable extract and milk with high reproducibility and reliability. This method serves as a convenient route to the rapid, sensitive, selective, and reliable detection of pathogens from complex food samples, with the potential to replace conventional yet laborious methods currently in use.

1. Introduction

Foodborne infections and diseases are becoming a pressing health concern with huge economic costs (Choi et al., 2018; Lee & Yoon, 2021; Nguyen & Kim, 2020). Among different foodborne pathogens, *Salmonella* species is one of the most prevalent pathogens that cause several illnesses even at low doses and can lead to eventual death without proper treatments (within 12–72 h) (Mirsadoughi et al., 2023; Wang et al., 2020). According to the World Health Organization (WHO), *Salmonella* is announced as one of the four leading causes of diarrheal

diseases worldwide (He et al., 2022). Among the plethora of *Salmonella* serotypes found in humans, *S. typhimurium* ranks first in the incidence rate, inducing food poisoning symptoms such as abnormal cramps, nausea, stomach ache, and diarrhea (Johnson et al., 2018; Wei et al., 2022). *S. typhimurium* has been demonstrated to possess strong pathogenicity by secreting several toxins, including endotoxins and enterotoxins, and extracellular pathogenic proteins (Yang et al., 2020). In addition, *S. typhimurium* exhibits high survival capacity in diverse living environments with a surprisingly low infection limit (approximately one colony-forming unit [CFU]). Most countries and regions have set strict

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regulations on *S. typhimurium* infection in food samples (Oslan et al., 2024). Thus, rapidly, timely, and effectively detecting the pathogenic bacterial infection in food is highly necessary (Shen et al., 2021; Silva et al., 2018).

Several methods, including culture-based approaches, nucleic acid amplification-based methods, and immunoassay through antigen–antibody interaction (e.g., enzyme-linked immunosorbent assay [ELISA]), have been conventionally used for detecting *S. typhimurium* (Du et al., 2022; Zheng et al., 2021). Although these methods can selectively and reliably diagnose *S. typhimurium* infection in food, they are still associated with limitations, such as inevitable utilization of sophisticated instrumentation by skilled operators, laborious and complex sample pretreatment/assay steps, and time-consuming processes (Rotariu et al., 2016; Yuan et al., 2014). In this regard, diverse biosensing strategies have been developed based on colorimetric, fluorescent, photothermal, magnetic, surface-enhanced Raman scattering (SERS), and electrochemical methods (Wei et al., 2022; Zhao et al., 2023). These techniques generally yield higher sensitivity in a shorter assay time than conventional methods. Most of the recent biosensors have been constructed utilizing the unique physicochemical characteristics of diverse nanomaterials composed of noble metals, including gold, silver, and platinum; metal oxides and chalcogenides, including manganese oxide, iron oxide, and cadmium selenide; and nanocarbons, including carbon dot and graphene oxide (Zhan et al., 2024; Zhang et al., 2020). Recently, dual- or triple-detection mode biosensors for *S. typhimurium* have been reported, providing high detection sensitivity with reliable multi-mode outcomes (Hu et al., 2018; Shen et al., 2022; Zhang et al., 2022). Although these methods effectively detect *S. typhimurium* infection in food, further advancement is still required to enable simpler and quicker, competitively selective, and sensitive detection—all of which are necessary to realize real-world applications.

Aptamers are single-stranded (ss) DNA or RNA molecules having selective affinity toward their target molecules (Lan et al., 2017; Yang et al., 2011). While some aptamers occur naturally, the majority are synthesized artificially through the systematic evolution of ligands by exponential enrichment (SELEX) method (Ni et al., 2020). With inherent assets such as high stability (both physical and chemical), facile synthesis, functionalization with other molecules, and broad categories of target, aptamers have increasingly been screened and employed as an alternative to conventional antibodies (Mahmoudpour et al., 2021; Xie et al., 2021). The implementation of aptamers in biosensing, known as aptasensors, has produced diverse measurable signals, such as optical, electrochemical, and mechanical, based on their interaction with employed nanomaterials, apparatus, and chromogenic dyes (Mao et al., 2020; Sharifi et al., 2020). Several aptasensors have been reported for the detection of *S. typhimurium*, with involvements of several nanostructures, such as graphene oxide, quantum dots, and gold nanoparticles, displaying the possibility for higher sensitive detection with relatively low cost (Wang et al., 2017; Fu et al., 2022; Duan et al., 2016; Fang et al., 2021). These approaches based on aptamers and nanostructures would open a new biosensing paradigm for developing rapid, sensitive, and cost-effective methods to detect *S. typhimurium* and other food pathogens (Zhou et al., 2021).

Graphitic carbon nitride (GCN) is an emerging nanomaterial due to its distinctive optoelectronic properties attributed to its high nitrogen content and π -electronic structure (Wang & Wang, 2022). GCNs are two-dimensional carbon-derived materials with a structural framework resembling graphene oxide's electronic configuration. As recently reported, GCNs possess fluorescence quenching capacity as well as peroxidase-like activity to oxidize chromogenic substrates, including 3,3',5,5'-tetramethylbenzidine (TMB), in the presence of H_2O_2 to generate colorimetric signals (Wang et al., 2019; Wang et al., 2017). However, their peroxidase-like activity is significantly low and insufficient for successful target detection. This deficiency was compensated for the modification on the surface of GCNs by ssDNA adsorption to boost their activity (Wang et al., 2017; Liu et al., 2021). Based on the

improved peroxidase-like activity when ssDNA adsorbed on GCNs, as well as their fluorescence quenching capacity, we describe a new and convenient colorimetric-fluorometric dual-mode method to detect *S. typhimurium*. In this strategy, a fluorescent dye 6-carboxyfluorescein (FAM)-labeled ssDNA aptamer toward *S. typhimurium* (Sal-Apt) was first incubated with GCNs to induce its adsorption primarily via a π - π interaction, yielding both increased peroxidase-like activity causing intense colorimetric signal and reduced fluorescence. When a sample containing target *S. typhimurium* was added to the mixture, FAM-labeled Sal-Apt detached from the GCN because of its higher affinity toward *S. typhimurium* than GCN. This event consequently reduced colorimetric signals and increased fluorescence proportional to the number of target bacterial species. The developed aptasensor can offer several user-friendly advantages, such as straightforward sample preparation, easy-to-read results (even with naked eye), and the dual-mode outcomes providing internal verification to reduce false positives and increase reliability. To our knowledge, this is the first study to develop a colorimetric-fluorometric dual-mode detection method for food pathogens using GCNs.

2. Experimental section

2.1. Materials

Tris base, melamine, cyanuric acid, dimethyl sulfoxide (DMSO), hydrochloric acid (HCl), 3,3',5,5'-tetramethylbenzidine (TMB), sodium hydroxide, sodium acetate, and phosphate-buffered saline (PBS) were purchased from Sigma-Aldrich (St. Louis, MO). Hydrogen peroxide (H_2O_2) was obtained from Junsei (Tokyo, Japan). UltraPure™ DNA/RNase-free distilled water was obtained from Invitrogen (Waltham, MA). Luria–Bertani (LB) broth was acquired from Becton Dickinson (Franklin Lakes, NJ). All other reagents and chemicals were of analytical grade or higher and were utilized without further purification. All solutions were prepared using ultrapure deionized (DI) water generated from a Milli-Q Purification system (Millipore; Darmstadt, Germany). Sal-Apt molecules with and without the fluorophore FAM were synthesized by Integrated DNA Technologies (IDT; Coralville, IA); these were 40-base ssDNA sequences, shown as follows (Oh et al., 2017):

5'-FAM-TATGGCGCGTCACCCGACGGGGACTTGACATTATGACA-3' and

5'-TATGGCGCGTCACCCGACGGGGACTTGACATTATGACA-3'.

2.2. Synthesis and characterization of GCNs and Sal-apt adsorbed GCNs

Rod-shaped GCNs were synthesized following our previous protocol using a melamine–cyanuric acid approach (Heo et al., 2020). The dried powder of synthesized GCN (50 mg) was further submerged in an HNO_3 solution (5 mM in DI water, 5 mL), followed by constant and intensive stirring for 1 day at room temperature (RT). The obtained particles were thoroughly washed with DI water until the pH of the solution reached neutral. The particles were further ultrasonicated for 2 h before being collected by centrifugation at 10,000g for 5 min. Finally, the particles were suspended in DI water and placed at 4 °C until use. Sal-Apt adsorbed GCN was prepared by mixing the FAM-labeled aptamer for *S. typhimurium* (Sal-Apt) (100 nM) with GCN (50 μ g/mL) and $MgCl_2$ (5 mM) in Tris-HCl buffer (1 mL, 10 mM, pH 7.4) and incubated for 30 min at RT in dark conditions under mild vortexing. After incubation, Sal-Apt-GCN was obtained by centrifugation at 10,000g for 5 min, followed by its suspension in the Tris-HCl buffer. The amount of aptamer in the supernatant was measured using a Nanodrop 1000 system (Thermo Fisher Scientific).

The morphologies and elemental compositions of bare GCNs and Sal-Apt adsorbed GCNs were analyzed by scanning electron microscopy (SEM; Magellan 400, CA) and transmission electron microscopy (TEM; ZEISS Libra 200 HT Mc Cs, Germany) equipped with energy dispersive X-ray spectroscopy (EDS). Zeta potential analyses were performed using

a Zetasizer Nono-ZS (Malvern Co.). Atomic force microscopy (AFM) was conducted using a JPK NanoWizard II bio-atomic force microscope (Bruker, MA) in the contact mode.

2.3. Bacterial culture

Bacterial strains, such as *S. typhimurium* (KCTC 2421), *Escherichia coli* (*E. coli* WT3110), *Staphylococcus aureus* (*S. aureus* KCTC 3881), and *Lactobacillus acidophilus* (*L. acidophilus* KCTC 3164), were grown in the LB medium at 37 °C at 200 rpm in a shaking incubator (SI-7820; Optima, Japan). The number of bacteria was determined using the plate counting method following incubation for 24 h at 37 °C followed by a serial dilution to calculate live cell concentrations. For colorimetric–fluorometric dual-mode detection experiments, the cultured bacteria were collected by centrifugation at 8000 g for 5 min, and the acquired cell pellets were washed thrice with DI water before being suspended in a sterile PBS buffer for further experiments.

2.4. Colorimetric–fluorometric dual-mode detection of *S. typhimurium* using Sal-apt adsorbed GCNs

The prepared Sal-Apt adsorbed GCNs served as a peroxidase mimic for colorimetric detection of *S. typhimurium*. In the assay, different concentrations of *S. typhimurium* suspensions were added into the reaction solution (100 μ L of Tris-HCl buffer, 10 mM, pH 7.4) containing Sal-Apt adsorbed GCNs (optimally at 0.1 mg/mL), followed by incubation at 37 °C for 10 min. Next, sodium acetate buffer solution (900 μ L, 10 mM, pH 4.0), including TMB (1 mM) and H₂O₂ (10 mM), was added to the mixture and incubated for 15 min at RT to induce peroxidase-like oxidation of TMB. After removing the GCNs and bacterial cells by centrifugation at 10,000 g for 5 min, the supernatant was used to evaluate the intensity of the generated blue color product corresponding to the oxidized TMB at 652 nm using a microplate reader (Synergy H1; BioTek, VT).

The prepared Sal-Apt adsorbed GCNs served as a quencher for the fluorescence of FAM-labeled Sal-Apt during the fluorometric detection of *S. typhimurium*. In the assay, different concentrations of *S. typhimurium* suspensions were added into the reaction solution (200 μ L of Tris-HCl buffer, 10 mM, pH 7.4) containing Sal-Apt adsorbed GCNs (optimally at 0.1 mg/mL), followed by the incubation at 37 °C for 10 min under dark conditions. Afterward, an aliquot of 100 μ L of the above solution was 10-fold diluted, and the fluorescence intensities were recorded with the excitation and emission wavelengths of 440 nm and 520 nm, respectively, in a black 96-well plate utilizing the microplate reader. Fluorescent images were acquired using a fluorescence imaging system (Kodak; Tokyo, Japan).

We next evaluated the practical utility of this method, for which different concentrations of *S. typhimurium* were spiked to food samples of vegetable extract and milk. Typically, pak choi (a type of cabbage) was purchased from a local grocery market and cleaned thoroughly using DI water to eliminate any potential interferences on the leafy surface. The samples (20 g) were chopped into 3 cm $\times$ 3 cm pieces using sterile scissors and subsequently soaked into 10 mL of Tris-HCl buffer (10 mM, pH 7.4), followed by incubation for 1 h under mild shaking conditions. The extract was obtained using a filter paper. Subsequently, the extracted samples were inoculated with pre-determined amounts of *S. typhimurium*. Milk was purchased and pretreated with conventional method (Mirsadoughi & Hosseini, 2023). Briefly, milk was filtered and centrifuged twice at 5000 g for 2 min to obtain the supernatant, and 20-fold diluted with Tris-HCl (10 mM, pH 7.4), which was used to inoculate *S. typhimurium* at concentrations of 100 and 10,000 CFU/mL. Finally, the number of *S. typhimurium* in each spiked sample was determined using the above-described procedures. Recovery rate (recovery [%] = measured value/added value $\times$ 100) and the coefficient of variation ([CV (%) = SD/average $\times$ 100] were determined to evaluate the precision and reproducibility of both colorimetric and fluorometric assays.

Stability of FAM-labeled Sal-Apt and GCN was assessed for one-week storage under different temperature conditions at –20 °C, 4 °C, and RT. First, the stability of FAM-labeled Sal-Apt was assessed by tracking its fluorescent intensity (FL) over storage time. Samples were kept at each temperature in dark conditions, and FL was recorded periodically. Similarly, GCN stability was assessed by evaluating its fluorescence-quenching efficiency of FAM-labeled Sal-Apt. FAM-labeled Sal-Apt adsorbed GCN complexes were stored at each temperature condition for one-week, and their quenching functionalities were measured periodically. In addition, colorimetric–fluorometric dual-mode assay was performed at day 1 and 7 using the same protocol to detect *S. typhimurium* at 10⁷ CFU/mL, to evaluate operational stability of Sal-Apt adsorbed GCN-mediated aptasensor.

3. Results and discussion

3.1. Preparation and characterization of FAM-labeled Sal-apt adsorbed GCNs

The principle of colorimetric–fluorometric dual-mode detection of *S. typhimurium* based on aptamer-adsorbed GCNs-mediated aptasensor is schematically illustrated in Fig. 1. Pristine GCNs exhibited weak peroxidase-like activity; however, the adsorption of FAM-labeled Sal-Apt on GCNs through π - π interaction dramatically bolstered their activity to oxidize TMB to generate an intense blue-colored product. This catalytic improvement is attributed to the negative charge of the adsorbed aptamer, enhancing the affinity toward the positively charged TMB substrate (Wang et al., 2017). In the presence of *S. typhimurium*, aptamers detach from GCNs to bind *S. typhimurium*, restoring the pristine state of GCNs and reducing the colorimetric signal due to reversal to their original peroxidase-like activity. The adsorption of FAM-labeled aptamer on GCNs simultaneously induced a significant reduction in FAM's fluorescence intensity due to the quenching effect of GCNs, which was derived from the photoexcited electron transfer from FAM to GCNs (Morales-Narváez & Merkoçi, 2019). In the presence of target species, FAM-labeled aptamer detaches from GCNs, thus recovering the fluorescence of FAM. Based on the two phenomena, the prepared GCN-based aptasensor could enable both signal-off colorimetric and signal-on fluorometric detection of *S. typhimurium*.

Pristine and FAM-labeled Sal-Apt adsorbed GCNs displayed a similar rod-shaped morphology, having length and width of approximately 100 and 20 nm, respectively (Fig. 2a, c). The aptamer-adsorbed GCNs were more aggregated than pristine GCNs, possibly by aptamer-mediated complex formation (Fig. 2b, d). AFM analyses of pristine and Sal-Apt adsorbed GCNs further confirmed the high level of aggregation of GCNs in the presence of aptamers (Fig. S1). EDS spectra revealed the presence of sulfur in FAM-labeled Sal-Apt adsorbed GCNs, which are generated due to the thiol linkage to conjugate FAM on the Sal-Apt, whereas the pristine GCNs did not have sulfur element (Fig. S2). These characterizations demonstrated that the FAM-labeled Sal-Apt adsorbed successfully on the GCNs.

Next, we evaluated the feasibility of achieving enhanced peroxidase-like activity and reduced fluorescence by adsorbing FAM-labeled Sal-Apt on GCNs. Pristine GCNs displayed very weak peroxidase-like activity to oxidize TMB, and as expected, aptamer adsorption significantly enhanced the peroxidase-like activity to yield the intense blue color signal (Fig. 3a). The enhanced colorimetric response was attributed to the improved electrostatic attraction between positively charged TMB and negatively charged aptamer, as demonstrated by the zeta potential analysis (Fig. S3) (Wang et al., 2017). FAM-labeled Sal-Apt displayed high fluorescence intensity, and the fluorescence was significantly reduced after its adsorption on GCNs due to GCN-mediated quenching (Fig. 3b). The quenching mechanism of GCN toward fluorophores was primarily followed the short-range photoinduced electron transfer (PET) mechanism, where the excited electron from a fluorophore being transferred directly to the electron-accepting surface of GCN due to a

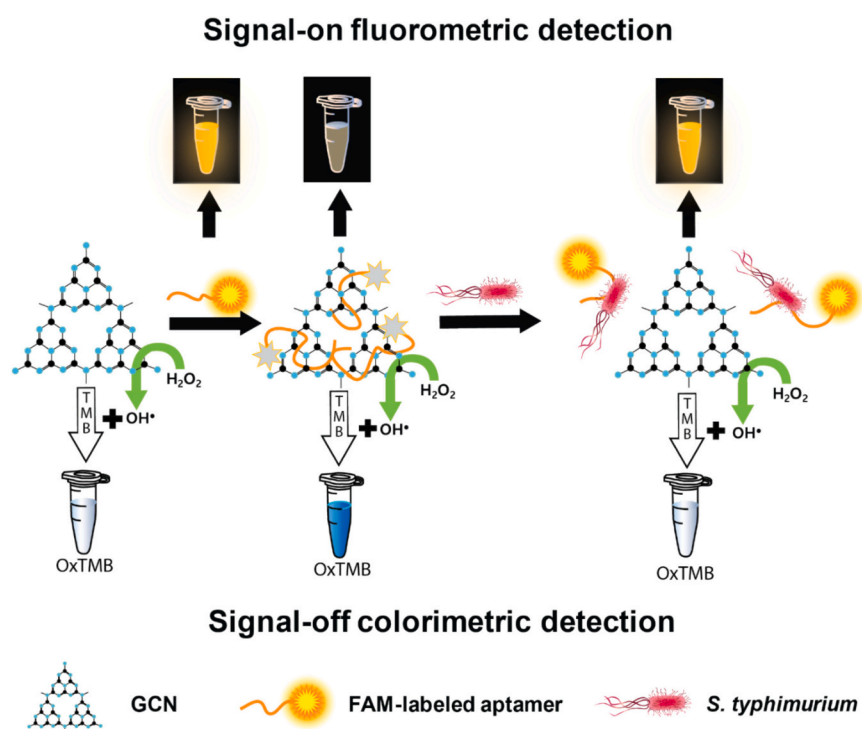


Fig. 1. Schematic illustration for colorimetric-fluorometric dual-mode detection of *S. typhimurium* using FAM-labeled Sal-Apt adsorbed GCNs.

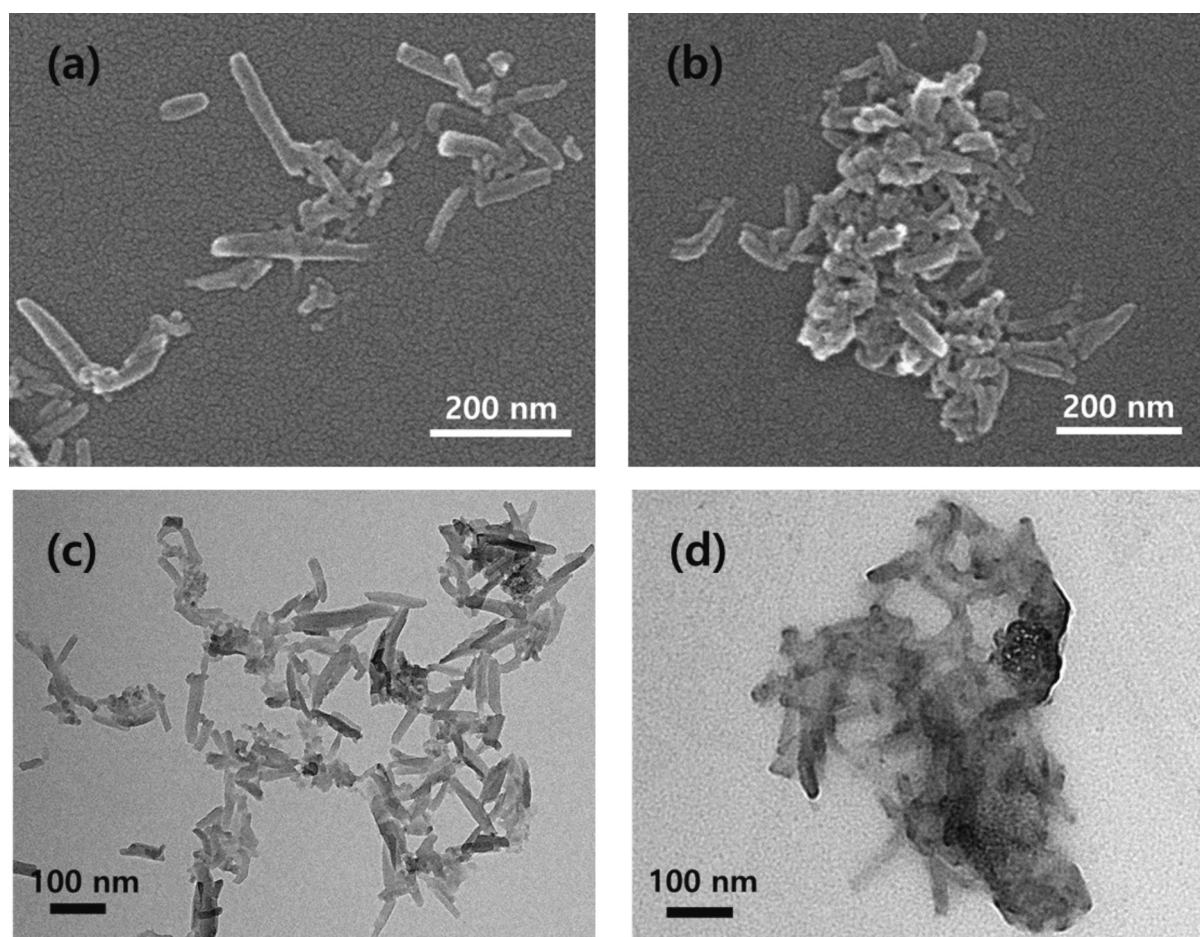


Fig. 2. SEM and TEM images of pristine GCNs [(a) and (c), respectively] and Sal-Apt adsorbed GCNs [(b) and (d), respectively].

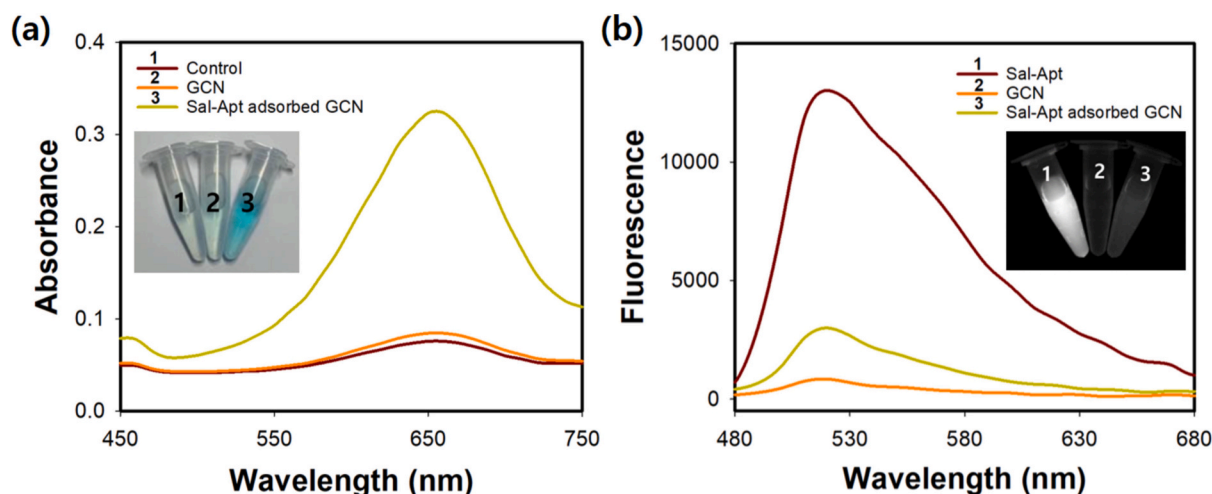


Fig. 3. (a) Absorption spectra with respective color change for TMB oxidation in the presence of H_2O_2 and (b) fluorescence spectra of GCN only and Sal-Apt adsorbed GCN.

very short distance between FAM fluorophore and GCN (Wang et al., 2013). It could be excluded from fluorescence resonance energy transfer (FRET) mechanism due to no overlap between emission spectrum of the donor and absorption spectrum of the receptor. With this fluorescent property, our system can detect target *S. typhimurium* based on the increased fluorescence through the detachment of FAM-labeled Sal-Apt from the GCNs in the presence of target.

Diverse conditions have been optimized to generate the most efficient FAM-labeled Sal-Apt adsorbed GCNs and assays for detecting *S. typhimurium*. We first examined the influences of several cationic ions, such as Na^+ , K^+ , Ca^{2+} , and Mg^{2+} , to optimize the fluorescence quenching capacity of GCNs (Owczarzy et al., 2008). The results demonstrated that divalent ions of Ca^{2+} and Mg^{2+} efficiently assisted in quenching the fluorescence (Fig. S4), and among them, Mg^{2+} ions were added in further experiments, similar to previous studies (Wang et al., 2019). As Mg^{2+} concentration increased, fluorescence quenching efficiency progressively improved with maximal value at 4 mM, probably due to the enhanced charge shielding and intraparticle bridging by the increased Mg^{2+} , which can bind more effectively to the functional groups on the GCN and increase its positive surface charge (Dong et al., 2019). Meanwhile, the fluorescence recovery was not significantly varied and just showed a slight decline at high Mg^{2+} concentration around 5 mM (Fig. S5a). In addition, the assay was not effective at basic pH conditions, presumably due to the increased electrostatic repulsion between GCN and Sal-Apt, as both surfaces would become more negatively charged at high pH conditions and thus yield reduced target binding and fluorescence recovery (Fig. S5b) (Choudhari et al., 2019; Tavassoli et al., 2023). The amounts of Sal-Apt and GCN were optimized by measuring the enhanced peroxidase-like activity and reduced fluorescence quenching efficiency (Fig. S6). Based on the observations, 100 nM FAM-labeled Sal-Apt, 50 μ g/mL GCN, 4 mM Mg^{2+} , and pH 7.4 were selected as ideal conditions and used for further preparation experiments.

3.2. Detection of *S. typhimurium* via colorimetric-fluorometric dual-mode strategies

The FAM-labeled Sal-Apt is expected to detach from GCNs in the presence of the target *S. typhimurium*, which may yield a reduced colorimetric signal and an enhanced fluorometric signal. The solution containing target *S. typhimurium* displayed a greatly reduced colorimetric response, which was similar to that of pristine GCN, via the typical assay with or without shaking environments for 30 min, to proceed target-specific aptamer detachment, TMB oxidation,

supernatant acquisition via centrifugation, and absorbance measurement (Fig. 4a). The extent of reduction was determined to be ca. 70 % based on a comparison of the absorption intensities at 652 nm. Significant reduction in the colorimetric intensity was observed toward target *S. typhimurium* (10^7 CFU/mL), whereas other bacteria, such as *E. coli*, *S. aureus*, and *L. acidophilus*, yielded a negligible reduction in the optical density, even at 10-fold higher concentrations (10^8 CFU/mL), indicating high specificity of the aptasensor (Fig. 4b). The high specificity was further confirmed using several mixed bacterial suspensions, demonstrating strong selectivity for *S. typhimurium* without any significant cross-reactivity (Fig. S7a). The absorbance intensity proportionally decreased with an increase in the concentrations of *S. typhimurium*, presumably due to a higher detachment ratio of Sal-Apt from GCNs. Thus, an inversely linear relationship between the absorption intensity and the logarithm of bacterial concentration was achieved from 10^7 to 10^1 CFU/mL with an R^2 value greater than 0.99 (Fig. 4c, d). Based on the calibration plot with the regression equation $y = -0.0401x + 0.3829$, in which y is the absorbance and x is the concentration of *S. typhimurium*, the limit of detection (LOD) was determined to be ~ 8 CFU/mL using the typical equation: $LOD = 3 \times \delta/k$, where δ is the standard deviation of blank and k is the slope of the calibration curve. These LOD and linear range values were among the best results of those recently reported for colorimetric *Salmonella* detection and sufficient to practically diagnose *S. typhimurium* infection (Table S1) (Du et al., 2022; Zhang et al., 2022).

In the fluorometric mode, we observed significant fluorescence enhancement over ca. 250 %, which almost reached that of the original intensity of FAM-labeled Sal-Apt, by just adding *S. typhimurium* into the reaction solution containing FAM-labeled Sal-Apt adsorbed GCNs with incubation for 10 min (Fig. 5a). This fluorescence recovery was attributed to a higher affinity for *S. typhimurium* rather than a consequence of physical disruption because the shaking condition did not exert any additional effects on the fluorescence recovery. Similar to the colorimetric mode, the fluorometric mode was specific toward *S. typhimurium*, whereas other bacterial strains did not induce any meaningful fluorescence recovery, even when used at 10-fold higher concentrations (Fig. 5b). Other bacterial strains did not interfere with the fluorescent signal generated by *S. typhimurium* in mixed bacterial suspensions, confirming high selectivity of the detection strategy (Fig. S7b). In addition, the fluorometric strategy allowed the detection of diverse concentrations of *S. typhimurium*, enabling a wide linear detection from 10^7 to 10^1 CFU/mL with an R^2 value greater than 0.99 (Fig. 5c, d). The LOD was determined to be as low as ~ 3 CFU/mL, which is one of the lowest compared to that reported previously (Table S1) (Fu et al., 2022; Hu et al., 2018). At 10^7 CFU/mL of *S. typhimurium*, approximately 90 %

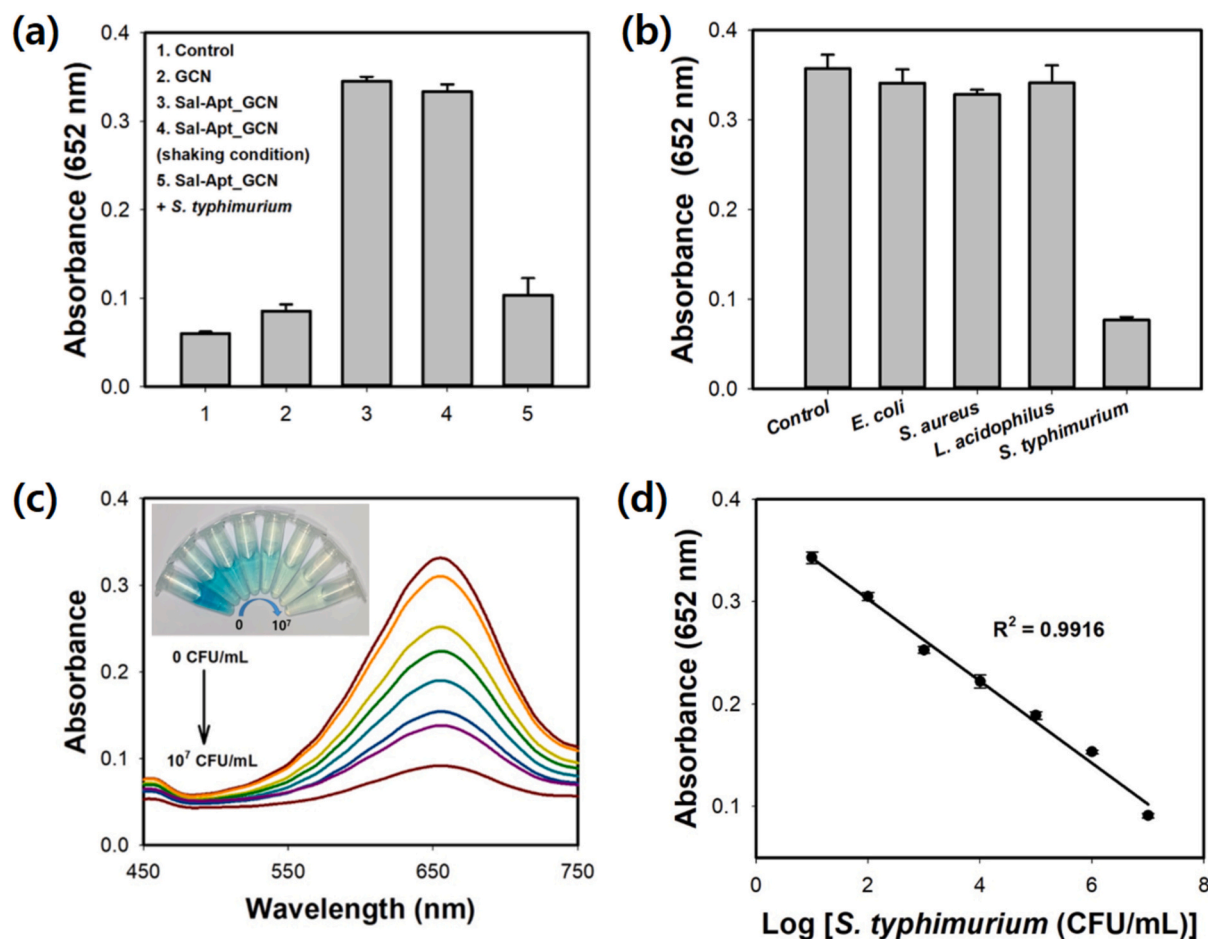


Fig. 4. (a) Absorption intensity of the assay solution obtained via TMB oxidation using Sal-Apt adsorbed GCNs (Sal-Apt GCNs) in the presence or absence of *S. typhimurium* (10^7 CFU/mL). (b) Selectivity of the signal-off colorimetric assay using Sal-Apt adsorbed GCNs toward *S. typhimurium*. (c) Absorption spectra and images of the reaction solution at different concentrations of *S. typhimurium* from 0 to 10^7 CFU/mL and (d) corresponding linear calibration plot.

of the initial fluorescence of FAM-labeled Sal-Apt was recovered, showing its near-perfect detachment from the GCNs (Fig. S8).

We observed that the adsorption of FAM-labeled Sal-Apt on GCNs yielded their marginal aggregation (Fig. 2b, d). We presumed that the FAM-labeled Sal-Apt would detach from GCNs in the presence of *S. typhimurium*. We further explored the mechanism for the *S. typhimurium*-induced detachment of aptamers by investigating the hydrodynamic sizes of pristine GCNs, Sal-Apt adsorbed GCNs, and Sal-Apt adsorbed GCNs after reacting with *S. typhimurium*. As shown in Fig. S9, Sal-Apt adsorbed GCNs were more aggregated than pristine GCNs, which was confirmed by ~3-fold larger hydrodynamic size. The aggregated Sal-Apt adsorbed GCNs were redispersed by the addition of target *S. typhimurium*, revealing that the introduction of *S. typhimurium* disrupted the aptamer-induced aggregation because of the higher affinity of the adsorbed Sal-Apt toward *S. typhimurium* than GCNs.

3.3. Practical utility to detect *S. typhimurium* in food sample

To demonstrate the practical utility, the developed dual-mode aptasensor was applied to detect *S. typhimurium* spiked in real food samples. Different concentrations of *S. typhimurium*, including 0, 100, and 10,000 CFU/mL, were spiked into the selected vegetable extract and milk samples, and applied to the developed GCN-based aptasensor. The aptasensor yielded excellent precision and reproducibility by achieving CVs of less than 3.17 % for both colorimetric and fluorometric modes and recovery rates of 102.9 to 95.5 % (colorimetry) and 105.7 to 98.4 (fluorometry) (Table 1). These results thus verified the promising

potential of the FAM-labeled Sal-Apt adsorbed GCN-based aptasensing platform in detecting *S. typhimurium* in complex food samples.

Practical applicability of the developed aptasensor was further highlighted through stability assessments of individual components (GCN and FAM-labeled Sal-Apt) and detection performances for *S. typhimurium*. The fluorescent intensity of FAM-labeled Sal-Apt and fluorescence quenching capacity of GCN successfully maintained over 90 % of their initial values during one-week storage under different temperature conditions (Fig. S10a, S10b). Furthermore, the detection capacity for *S. typhimurium* using both colorimetric and fluorometric modes was not varied during one-week storage (Fig. S10c, S10d). These results demonstrate the high stability of the developed aptasensor, which is quite beneficial for realizing practical applications in fields.

4. Conclusions

In conclusion, we established a new colorimetric–fluorometric dual-mode methodology for detecting *S. typhimurium*. The method depends on the target bacteria-induced reduction in the colorimetric signal and enhancement in the fluorometric signal through the detachment of Sal-Apt from GCNs. The significance of this study is that a highly simplified yet rapid method for sensitive and credible dual-mode detection of *S. typhimurium* was developed utilizing the unique interaction between FAM-labeled aptamer and GCNs. This novel strategy can be utilized to analyze the samples down to ~8 CFU/mL by colorimetry within 30 min or ~3 CFU/mL by fluorometry within 10 min, all without the complicated pre-and post-assay steps and instrumentations often required

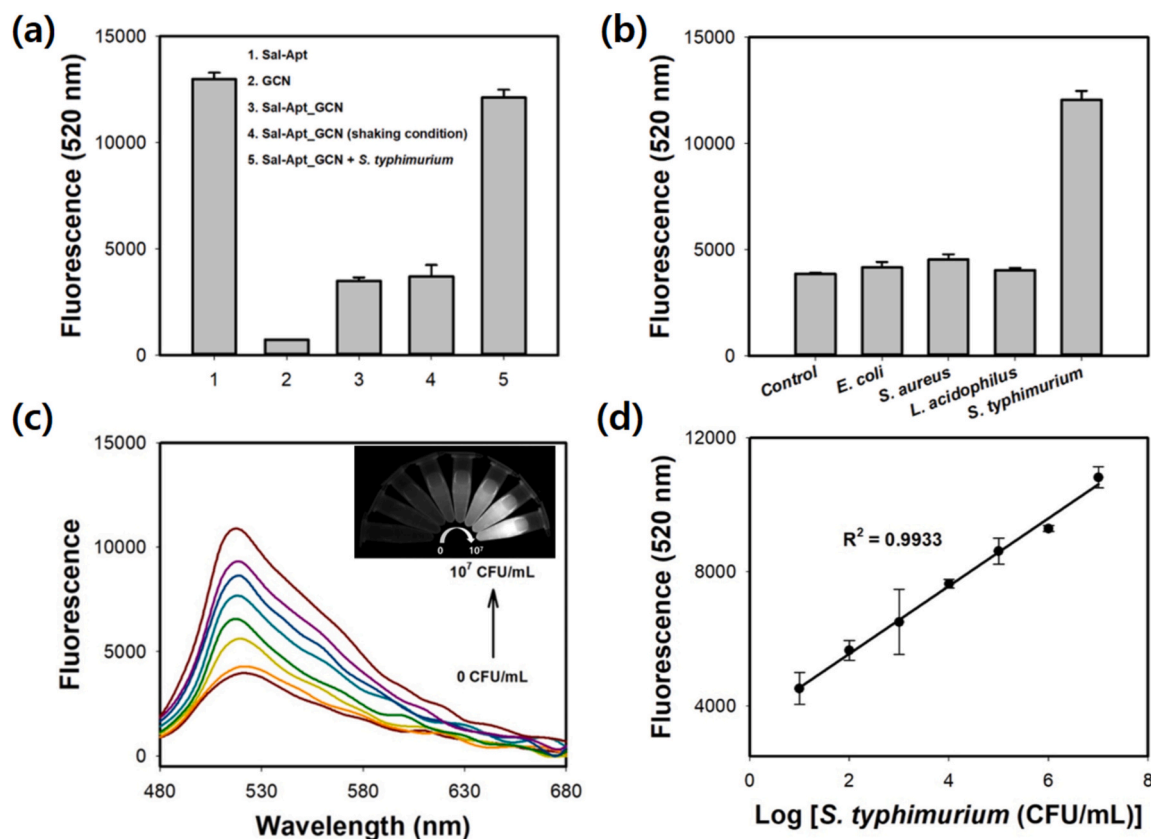


Fig. 5. (a) Fluorescence intensity of the assay solution using Sal-Apt adsorbed GCNs (Sal-Apt GCNs) in the presence or absence of *S. typhimurium* (10^7 CFU/mL). (b) Selectivity of the signal-on fluorometric assay using Sal-Apt adsorbed GCNs toward *S. typhimurium*. (c) Fluorescence spectra and images of the reaction solution at different concentrations of *S. typhimurium* from 0 to 10^7 CFU/mL and (d) corresponding linear calibration plot.

Table 1

Detection precision of the Sal-Apt adsorbed GCN-mediated colorimetric-fluorometric dual-mode strategy for *S. typhimurium* spiked in food samples.

Method	Sample	Spiked (CFU/mL)	Detected ^a (CFU/mL)	Recovery ^b (%)	CV ^c (%)
Colorimetry	Vegetable	1 0	ND ^d	—	—
		2 100	96	95.5	0.87
		3 10,000	9716	97.2	1.16
	Milk	1 0	ND	—	—
		2 100	98	98.3	1.40
		3 10,000	10,291	102.9	1.60
Fluorometry	Vegetable	1 0	ND	—	—
		2 100	106	105.7	1.47
		3 10,000	9840	98.4	2.67
	Milk	1 0	ND	—	—
		2 100	104	103.9	3.17
		3 10,000	10,416	104.2	2.16

^a Average value of five independent measurements.

^d Recovery = (detected value/spiked value) $\times$ 100.

^c Coefficient of variation = (SD/average) $\times$ 100.

^b ND: not detected.

while using time-consuming traditional approaches (several hours to days). The practical utility of this dual-mode detection strategy was successfully demonstrated by its application in detecting *S. typhimurium* spiked in vegetable extract and milk. Due to its beneficial properties including affordable materials and reagents, easy-to-follow procedures, and rapid assay time, the methodology should be extensively used as a novel aptasensor for the detection of target bacteria in complex food samples.

CRediT authorship contribution statement

Thinh Viet Dang: Writing – original draft, Investigation, Conceptualization. **In Seung Jang:** Writing – original draft, Investigation. **Quynh Huong Nguyen:** Investigation, Conceptualization. **Hyeun Seok Choi:** Methodology. **Byung Jo Yu:** Writing – review & editing, Conceptualization. **Moon Il Kim:** Writing – review & editing, Supervision, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2024.142176>.

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