1. Experimental

1.1 Materials

Graphene oxide was purchased from Xianfeng Nano (Nanjing, China). All the DNA probes were synthesized by Shanghai Sangon Biological Engineering Technology (Shanghai, China). The following oligonucleotides were used in this study:

FAM modified S-aptamer: 5'-FAM-TCGGCAACAAGGTCACCCGGAGAAGATC GGTGGTCAAACTGCATAGGTAGTCCAGAAGCC-3'

Cy3 modified E-aptamer: 5'-Cy3- ATCAAATGTGCAGATATCAAGACTGTA CAAGAT-3'.

The classical strain of S. *enteritidis* (ATCC 13076), S. *Paratyphi A* (isolated from patients infected with Salmonella), Salmonella *Cholerae-suis* (ATCC10708), and *E. coli*, ETEC K88 (CVCC 216) were obtained from the Guangzhou institute of microbiology (Guangzhou, China). All the bacteria were cultured at 37°C in Nutrient-Broth medium (peptone 10 g/L, beef extract powder 3 g/L, NaCl 5 g/L) with shaking at 100 rpm. Bacteria at the early log growth phase (OD600 of about 2.1) were used in the detection. The bacteria were collected by centrifugation at 1,000 × g for 15 min at 4 °C, followed by three times of washing with phosphate buffered saline (PBS). The number of bacteria were determined via the Luria-Bertani plate count method (0.1% Trypton, 0.5% Yeast extract,1.0% NaCl, 1.5% Agarose).

Strains were cultured aerobically for 16 h at 37°C in 50 mL of Luria-Bertani (LB) medium (final OD>1 for each sample). Then, the bacteria were serially diluted in PBS. For colony formation test, 500 μL of the bacteria suspension were diluted and plated on dishes with LB agar in triplicate. The dishes were incubated at 37°C for 24 hours to allow bacterial growth. The colonies were then counted. The number of colony forming units (CFUs) was a mean of three experiments.

All other reagents were purchased from standard commercial sources and were of analytical grade. All buffer solutions used in this study were prepared in our laboratory using ultrapure water (resistance of 18MΩ).

1.2 Procedure of pathogen detection

The pathogen detection was conducted as followings. Firstly, graphene oxide (20 mg/mL) and FAM modifed S-aptamer (100 μM) were added into 500 μL of PBS, mixed gently and incubated at room temperature for 20 min. Secondly, a certain amount of S. *enteritidis* was added into the GO/Aptamer solution. After 10 min of incubation, the fluorescence recovery with an emission wavelength at 520 nm was recorded by a fluorescence spectrophotometer (LS55, Perkin–Elmer, Hayman, Germany).

1.3 Performance of aptasensor

Selectivity of the assay was firstly tested using 3 strains of bacteria available for this study. The FAM modified aptamers (200 nM) were incubated with S. *Paratyphi A*, *E. coli* K88, S. *enteritidis*, and S. *Cholerae-suis* (10^6 CFU/mL each) in 500 μL of PBS, respectively. The fluoresence recovery in each bacteria group was monitored. Sensitivity of the aptasensor was tested using a serial dilutions of S. *enteritidis*. The fluoresence recovery was then monitored by a fluorescence spectrophotometer.

In order to test the performance of this aptasensor in real sample detection, we challenged this aptasensor with skim milk. Firstly, take 200 μl bacterial suspension of a certain concentration to mix with 1 ml of 3% milk. Secondly, 600 μl of the suspension was used for detection with our aptasensor. According to the equation of *Log*[concentration of S. e*nteritidis*] *VS* fluorescence intensity, the concentration of bacterial was calculated (calculated concentration). Another 600 μl of the suspension was plated on LB agar dishes to form colonies (known concentration). The recovery rate=calculated concentration/known concentration×100%.

In the multiplex detection, two pairs of fluorescein modified aptamer, E-aptamer and S-aptamer were used. E-aptamer specific for *E.coli* was modified with Cy3 and S-aptamer specific for S. *enteritidis* was modified with FAM. The two aptamers were firstly incubated with GO to form the GO/Aptamer complexes. Then, two strains of bacteria, *E.coli* and S. *enteritidis,* were added into the GO/Aptamer solution. The fluorescence recovery at two different emission wavelengths (520 nm for FAM and

570 nm for Cy3) was recorded individually. Furthermore, S. *enteritidis* was spiked in 3% skim milk for the real sample detection.

1.4 Statistical analysis

SPSS 10.0 software (SPSS Inc., IL, US) was used for statistical analysis. The results were expressed as means±SD of three independent experiments. Individual comparisons were made by Student's t-test for paired data and *P*-values less than 0.05 were considered to be significant.

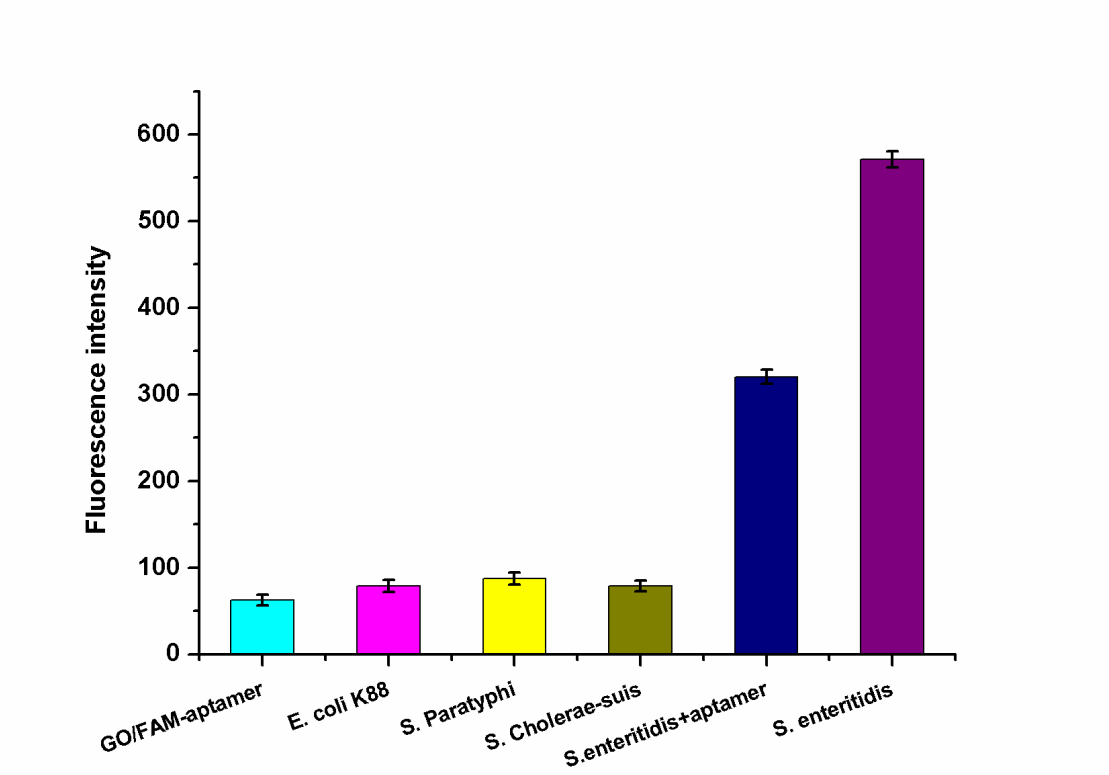

Figure S1. Selectivity of the GO-based aptasensor for S. *enteritidis* detection. From left to right: GO+FAM-aptamer only, E.*coli* K88, S. *Paratyphi*, S. *Cholerae-suis*, S-aptamer+ S. *enteritidis* and S. *enteritidis* were added and then the fluorescence intensity at 520 nm were recorded. Error bars indicate standard deviations from three independent experiments.

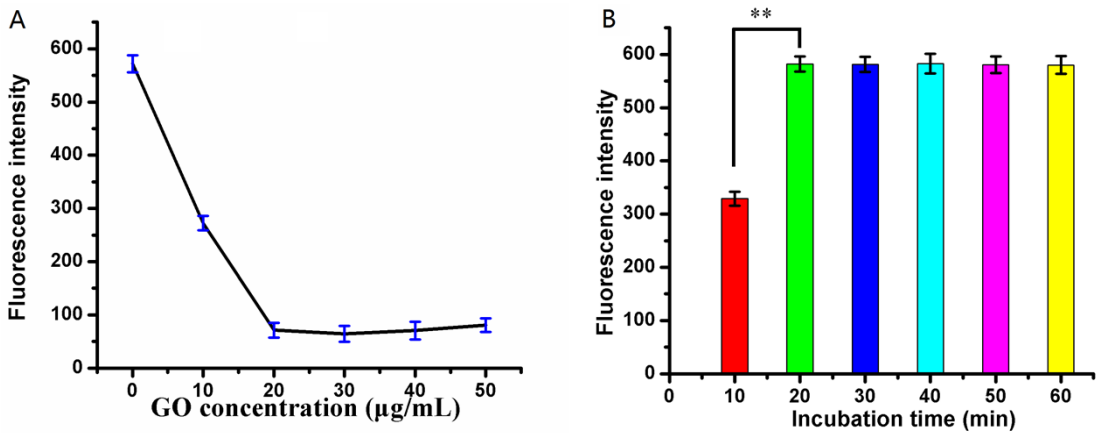

Figure S2. Optimization of experimental parameters. A: Fluorescence quenching using different concentrations of GO; B: The fluorescence recovering at different incubation time. Error bars indicate standard deviations from three independent experiments.