Supporting Information

Introduction of multilayered fluorescent nanofilm into lateral flow immunoassay for ultrasensitive detection of *Salmonella typhimurium* in food samples

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S1. Experimental section

S1.1 Materials, chemicals, and instruments

N-(3-dimethyaminopropy)-N′-ethylcarbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide sodium salt (sulfo-NHS), bovine serum albumin (BSA), 2-(N-morpholino)ethanesulfonic (MES), fetal bovine serum (FBS), and polyethyleneimine branched (PEI, MW 25 kDa) were purchased from Sigma-Aldrich (USA). Nitrocellulose membrane (UniSart CN95) with 15 μm pore size was obtained from Sartorius (Spain). Mouse monoclonal antibody to *S. typhi* (catalog no. ab8274) was purchased from Abcam (Cambridge, U.K.). Goat anti-mouse IgG was purchased from Sangon Biotech Co., Ltd. (Shanghai, China).

Monolayer graphene oxide (Catalog #100682, 200–500 nm) was purchased from XFNANO, Inc. (Nanjing, China). CdSe@ZnS-COOH QDs (excitation/emission

maxima ~ 365/631 nm) were supplied by Mesolight Inc. (Suzhou, China). Carboxylic group-modified CdSe/ZnS core–shell QBs (emission at 605 ± 5 nm) were purchased from the Beijing Najing Biological Technology Co., Ltd. (Beijing, China). The sample loading pad, conjugate pad, absorbent pad, and plastic backing card were purchased from Jieyi Biotechnology Co., Ltd. (Shanghai, China).

The TEM images of fabricated GO-based fluorescent nanofilms were taken on Tecnai G2 F20 microscope. Elemental mapping images were recorded by EDS spectroscopy using a Philips Tecnai G2 F20 microscope equipped with a STEM unit. Zeta potential was determined using a Mastersizer 2000 (Malvern, UK). Fluorescence signals of the GO/DQD-based LFIA strips were measured on a portable FIC-S1 fluorescent strip reader (Suzhou Hemai, China).

S1.2 Bacteria sample preparation

The standard strain of pathogens used in this study were provided by Prof. Bing Gu from Laboratory Medicine, Guangdong Provincial People's Hospital, and the concentration of these bacteria was verified by traditional plate counting. Briefly, the bacterial strain was inoculated into 5% sheep blood agar plates at 37 °C in an incubator containing 5% CO_2 overnight. Several colonies were directly isolated from the plates and transferred into 1 mL of sterile PBS solution (10 mm, pH 7.4). The as-prepared bacterial solution was diluted 1×10^5–1×10^8 times with sterile water, and 0.1 mL of the diluted sample was coated to blood agar plates for 12 h. The colony forming units (CFUs) on the plates were counted to determine the concentration of the original bacteria samples. Finally, the prepared *S. typhi* samples (10^6–10 cells/mL) with defined concentration were prepared by dilution of the original bacteria solution.

S1.3 Preparation of AuNP-based LFIA strips

First, we prepared 30 nm Au NPs using a conventional trisodium citrate reduction method. Second, the pH of 10 μg of *S. typhi* antibody was adjusted to 9 with 0.2 M K_2CO_3 and incubated with 1 mL 30 nm AuNP (pH 9) for 15 min. Then, 100 μL of BSA solution (10 mg/mL) was added to block the unreacted sites of AuNPs. The as-prepared immuno-AuNPs were separated by centrifugation (4500 rpm, 6 min), and resuspended with 200 μL of storage solution (10 mM PB solution containing 1% BSA

(w/v), 0.1% PVP (w/v), 10% sucrose (w/v), 0.02% NaN_3 (w/v), and 0.05% Tween-20 (v/v)). Finally, the antibody-conjugated AuNPs were directly mixed with the bacteria sample and dropped onto the sample pad of LFIA.

S1.4 Preparation of QB-based LFIA strips

The spherical QB was prepared according to our previously proposed method (Zhang et al., 2020). The conjugation of QB and anti-*S. typhi* antibody was conducted via the carbodiimide chemistry. First, 100 μL of QB was added in 100 μL MES buffer (100 mM, pH 6.0), and then mixed with 5 μL of EDC (10 mM) and 10 μL of sulfo-NHS (10 mM). After activation for 15 min, the mixture was centrifuged to remove the excess activators (EDC/sulfo-NHS) and dispersed in 100 μL PBS buffer (10 mM, pH 7.4). Then, the activated QB solution was incubated with 10 μg of *S. typhi* antibody for 1 h at the temperature of 37°C, followed by surface blocking with 50 μL 1% BSA solution for 30 min. The antibody-conjugated QBs were separated by centrifugation (8000 rpm, 6 min), and resuspended with 200 μL of storage solution (10 mM PBS containing 1% BSA (w/v), 0.5% sucrose (w/v), 0.02% NaN_3 (w/v)). Finally, the antibody-conjugated QBs were directly diluted and mixed with the bacteria sample and dropped onto the sample pad of LFIA.

References

Zhang, B., Yang, X., Liu, X., Li, J., Wang, C., Wang, S., 2020. Polyethyleneimine-interlayered silica-core quantum dot-shell nanocomposites for sensitive detection of Salmonella typhimurium via a lateral flow immunoassay. RSC Advances 10(5), 2483-2489.

S2. Calculation of the maximum number of QDs on the GO/DQD nanofilm

On the basis of the surface distribution of CdSe/ZnS QDs on GO nanosheets (Fig. 4d), we estimated the maximum average number of QDs on the surfaces of GO-DQD nanofilms by using the following model:

Model description

Some spherical QDs with the diameter of 12 nm are adsorbed onto the surface of a square GO nanosheet with the length of 600 nm. However, a gap of at least 8 nm is

present between each QD. We want to know the maximum number of small QDs that can be absorbed on the surfaces of the GO nanosheet (both sides are absorbent).

The model can be simplified into a model like the one shown in Fig. 4e. A square with the length of L (= 600 nm) is covered with small circles with the diameter of a (= 12 nm) with a gap with the length of b (= 20 nm) between each circle. Let x denote the maximum number of balls that are arranged horizontally and y denote the maximum number of balls that are arranged vertically. Then, the following inequalities need to be satisfied at the same time:

$$\begin{cases} ax + b(x-1) \leqq L \\ a + \frac{\sqrt{3}}{2}(a+b)(y-1) \leqq L \end{cases}$$

Let $L = 600\ nm\ and\ a = 12\ nm$. Then, the results are calculated. x_{max}= 19, y_{max}= 21.

Therefore, the GO nanosheet can adsorb (19 × 11 + 18 × 10) × 2 = 778 small spherical QDs at most.

Obviously, the loading amount of QDs can be doubled by adding one more layer of the PEI-QD shell.

Thus, the loading amount of QDs onto GO/DQD nanosheet can be calculated as 1556.

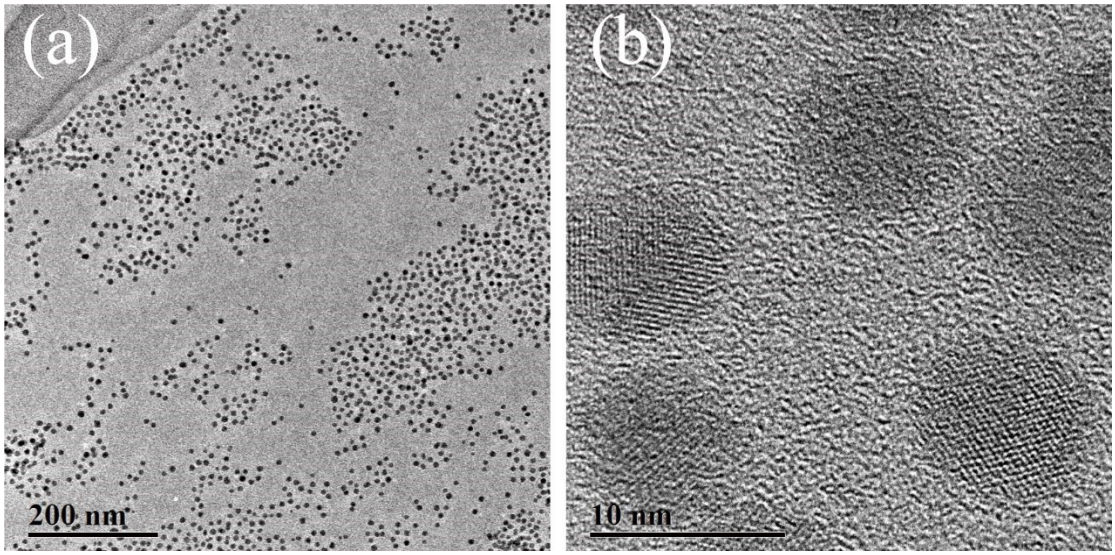

Fig. S1 (a) TEM and (b) corresponding enlarged TEM images of 12 nm CdSe/ZnS-MPA QDs.

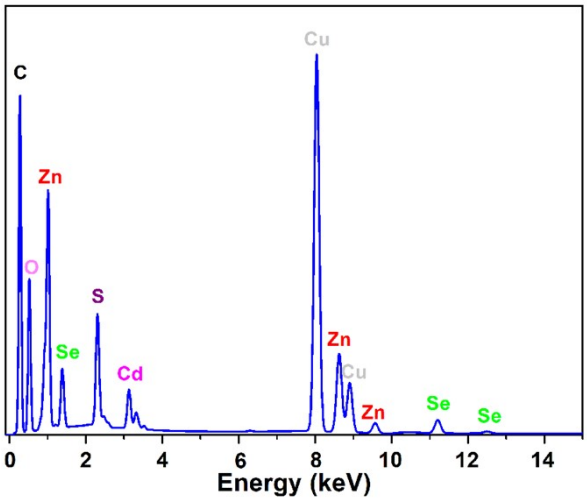

Fig. S2 EDS data from a single GO/DQD nanofilm. The Cu signal is from the Cu grids of the TEM sample.

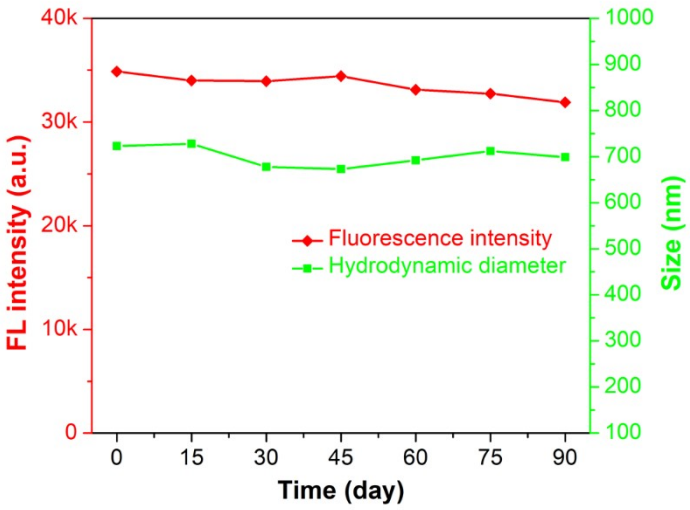

Fig. S3 Hydrodynamic diameter (green line) and fluorescence intensity (red line) of GO/DQD nanofilm stored in ethanol against the storage time.

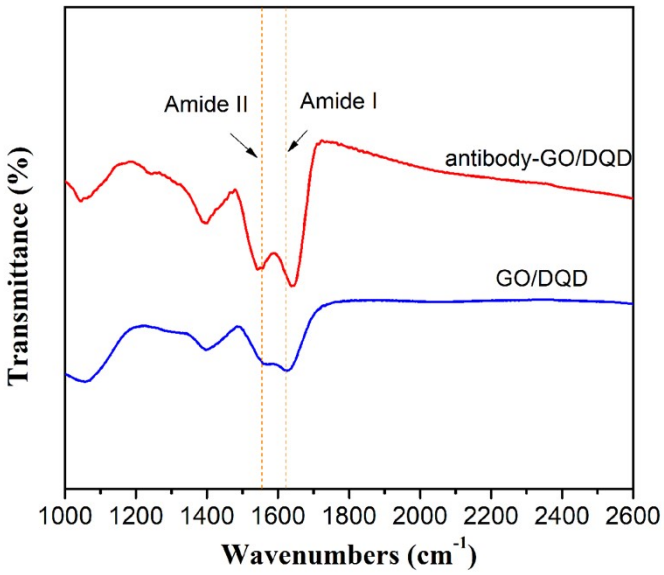

Fig. S4 FTIR spectra of GO/DQD (blue line) and antibody-modified GO/DQD (red line). The characteristic absorption peaks corresponding to protein amide bands I (1641 cm^{-1}) and II (1530 cm^{-1}) appearing in immuno-GO/DQD reveals the success of coupling.

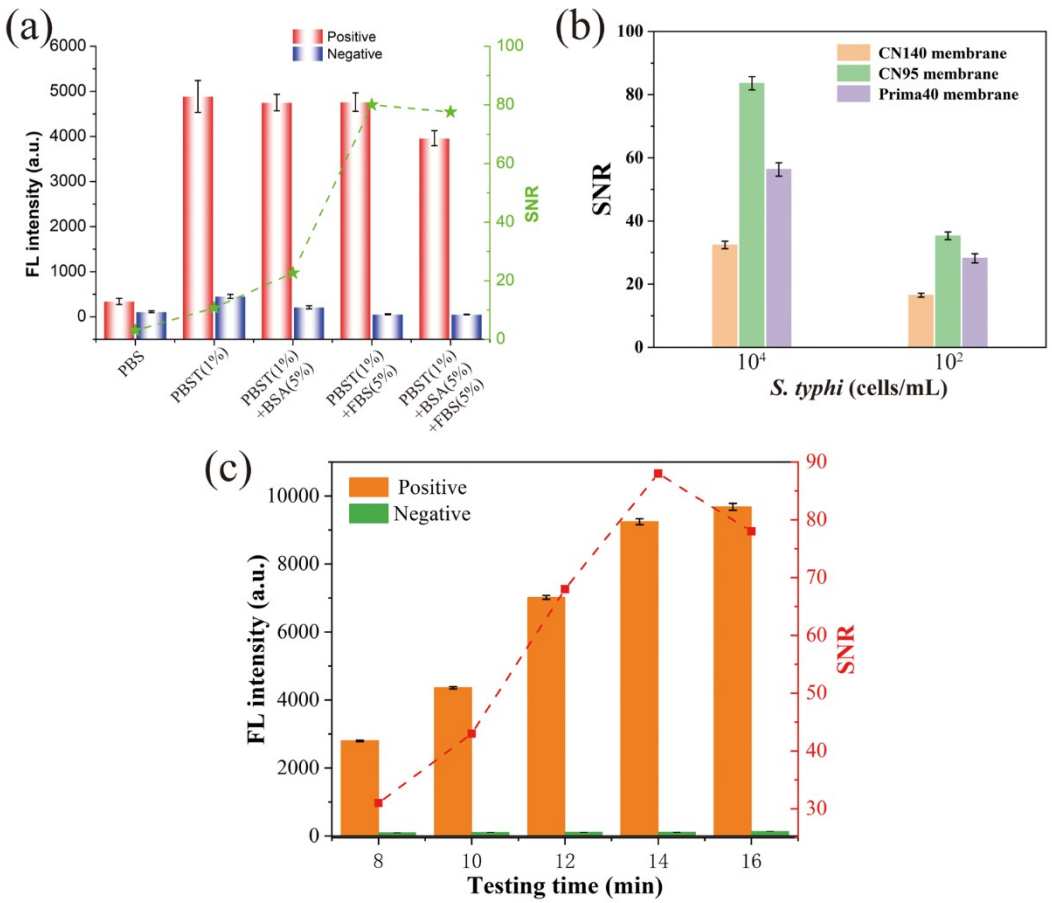

Fig. S5 Optimization of (a) running buffer, (b) NC membranes and (c) chromatographic time for GO/DQD-LFIA strip. The error bars indicate standard deviations calculated from three measurements.

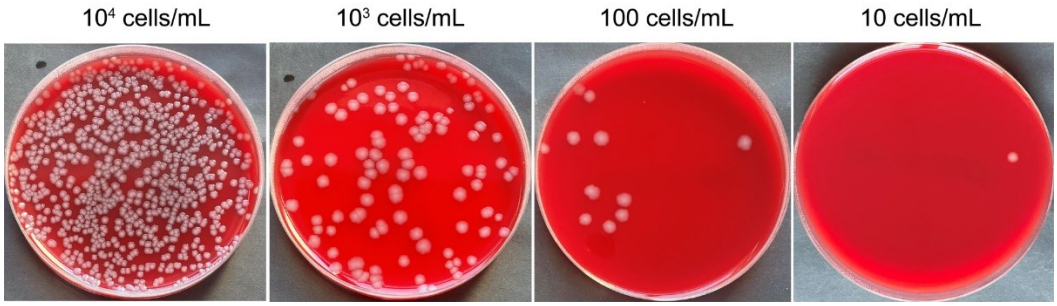

Fig. S6 Plate counting results for the prepared *S. typhi* samples.

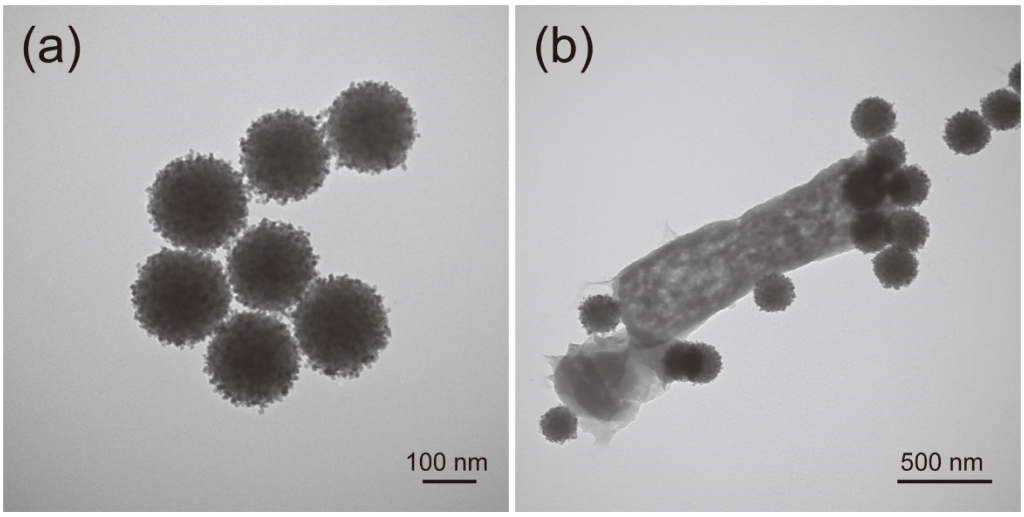

Fig. S7 TEM images of (a) spherical QB tags and (b) spherical QB-*S. typhi* immunocomplexes.

Table S1. *S. typhi* detection results of GO/DQD-LFIA in spiked real food samples.

Sample	Added *S. typhi* (cells/mL)	Detected *S. typhi* (cells/mL)	Recovery (%)	RSD (%)
Meat extraction	1×10^4	9.38×10^3	93.8%	9.7%
	1×10^2	95	95.0%	6.9%
Egg extraction	1×10^4	9.06×10^3	90.6%	9.2%
	1×10^2	89	89.0%	10.6%
Cabbage extraction	1×10^4	1.32×10^4	132.0%	12.4%
	1×10^2	121	121.0%	7.5%