

Supplementary Materials

A Microfluidic Biosensor Based on Magnetic Nanoparticle Separation, Quantum Dots Labeling and MnO₂ Nanoflower Amplification for Rapid and Sensitive Detection of *Salmonella* Typhimurium

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1. Preparation of the bacteria

In this study, *Salmonella* Typhimurium (ATCC 14028) was used as target bacteria, *E. coli* O157:H7 (ATCC 43888), *Listeria monocytogenes* (ATCC13932), *Staphylococcus aureus* (CICC10001), *Bacillus cereus* (CICC10041) and *Vibrio cholera* (CICC23795) from China Center of Industrial Culture Collection (CICC) were used as the non-target bacteria for evaluating the specificity of the proposed biosensor. All bacteria were stored at −20 °C with 15% glycerol, which were first revived in Luria-Bertani (LB) medium at 37 °C with shaking at 180 rpm for 12 hours respectively. Then, they were serially 10-fold diluted with the sterile PBS to obtain concentrations from 10¹ to 10⁶ CFU/mL.

2. Preparation of the MnO₂ NFs

Briefly, 100 mL 0.1 mol/L of KMnO₄ solution and 0.0261 g of polyvinyl pyrrolidone (PVP) were dissolved in 10 mL of distilled water under vigorous stirring, then HCl (100 mL, 0.2 M) was added into the mixture when the temperature rose to 90 °C. The precipitates were synthesized and cooled to room temperature after reacting for 1 h. Then, the precipitates were washed with distilled water for several times and dissolved in deionized water. The MnO₂ NFs were stored at 4 °C for further use.

3. Preparation of the MnO₂ NFs

Different amounts of quantum dots (QDs) were used to synthesize the MnO₂-QD NFs. The QDs were released by the same glutathione (GSH) and measured to optimize the amount of QDs. As shown in Figure S2a, the mean fluorescent intensity of the released QDs increases from about 300 counts to about 800 counts when the amount of QDs changes from 0 to 50 pmol. Further increase on the amount to 70 pmol does not result in obvious increase on the intensity. Thus, the optimal amount of 50 pmol for the QDs was used to synthesize the MnO₂-QD NFs in this study.

Different amounts (MnO₂ NFs: from 0.02 mg to 0.06 mg) of the MnO₂-QD-pAb NFs were used to detect the target bacteria with the concentration of 1.0 × 10⁵ CFU/mL at the optimal conditions and the fluorescent intensity of the released QDs was measured at the characteristic wavelength of 661 nm. As shown in Figure S2b, the mean fluorescent intensity increases from about 200 counts to about

560 counts when the amount of the MnO₂-QD-pAb NFs changes from 0 µg to 50 µg, and the intensity remains at the same level when the amount further increases to 60 µg. Thus, the optimal amount of 50 µg for the MnO₂-QD-pAb NFs was used in this study.

The MnO₂-QD-pAb NFs and the mixture of the immune MNPs and the target bacteria at the concentration of 1.0×10^5 CFU/mL were injected into the microfluidic chip with different flow rates from 20 µL/min to 40 µL/min at the optimal conditions. As shown in Figure S2c, the mean fluorescent intensity increases from about 400 counts to about 670 counts when the flow rate decreases from 40 µL/min to 25 µL/min and does not increase obviously when the flow rate further decreases to 20 µL/min. Thus, the optimal flow rate of 25 µL/min was used in this study.

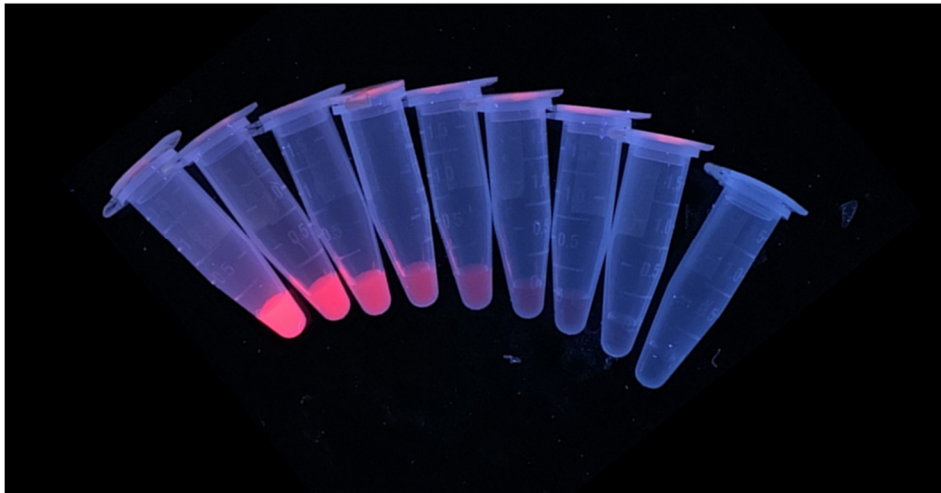
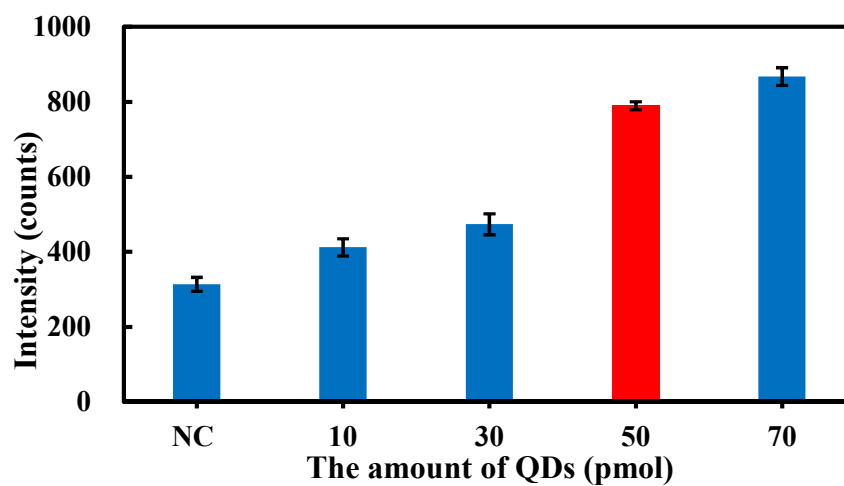
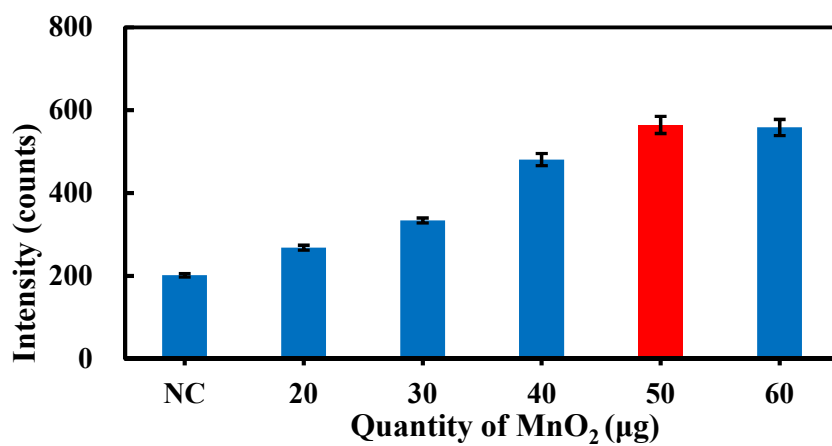


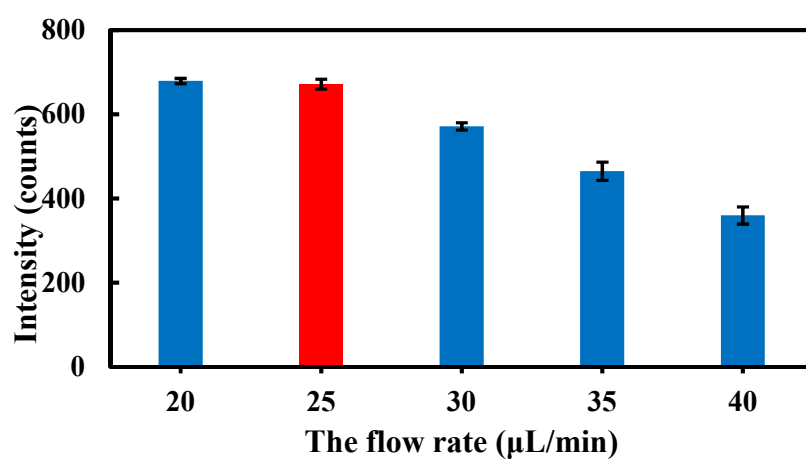
Figure S1. The fluorescence of the released quantum dots (QDs) on 2-fold dilutions of the MnO₂-QD NFs.



(a)

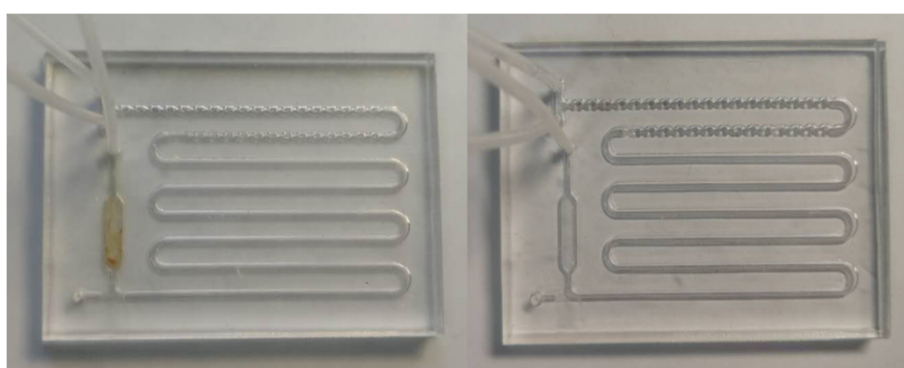


(b)



(c)

Figure S2. (a) Optimization of the amount of the QDs; (b) Optimization of the amount of the MnO₂-QD-pAb NFs; (c) Optimization of the flow rate.



(a)

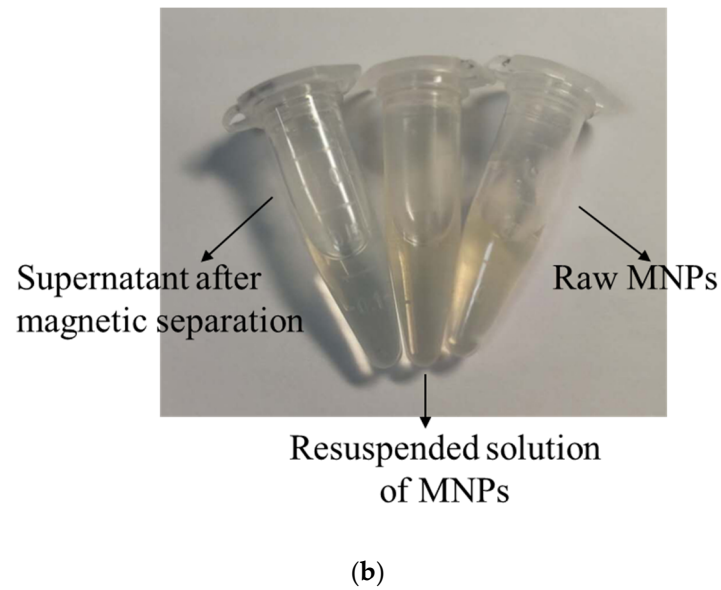


Figure S3. (a) Difference in separation chamber with or without magnets; (b) The color of supernatant of magnetic nanoparticles (MNPs), suspended MNPs after separating and original MNPs.



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