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A dual amplification strategy for DNA detection combining bio-barcode assay and metal enhanced fluorescence modality

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Experimental Procedures

1. Chemicals and Instrumentation

All nucleotides were synthesized and HPLC-purified by Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequence information as indicated in the specific experiment has been provided in the Supporting Information. Silver nitrate (AR), trisodium citrate (AR), sodium chloride (AR), sodium dihydrogen phosphate (AR), disodium hydrogen phosphate (AR), polyethylene glycol (PEG-400), and disodium tetraborate (AR) were purchased from Beijing Chemical Plant (Beijing, China). Sodium borohydride (AR) was from Tianjin Xuan'ang Co. Ltd. (Tianjing, China), and ascorbic acid (AR) was from Shanghai Chemical Reagent Plant (Shanghai, China). Tris(2-carboxyethyl)phosphine (TCEP) (≥ 99%) was from Sangon Biotechnology Co., Ltd. (Shanghai, China). Iron(III)chloride hexahydrate (AR) was from Sinofarm Chemical Reagent Co., Ltd. All other chemicals, unless mentioned otherwise, were of analytical grade (AR). Wahaha® purified water was used throughout the study.

Phosphate buffer (pH 7.5, 200 mM) was prepared by mixing 84.0 mL of 0.2 M Na_2HPO_4 with 16.0 mL of 0.2 M NaH_2PO_4.

Fluorescence spectra were recorded with a Hitachi F-4500 spectrofluorometer (Tokyo, Japan). Fluorescence spectra were scanned with a synchronous mode at $\Delta\lambda$ = 23 nm (λex/em=495 /518 nm) to avoid scattered light from the suspension (PMT : 700 V; slit(ex/em): 5/5 nm). Extinction spectra of AgNPs were obtained with a Hitachi U-3010 spectrophotometer (Tokyo, Japan). Transmission Electron Microscopic (TEM) images were acquired with a JEOL-200CX transmission electron microscope (Tokyo, Japan). For the TEM measurement, a 5-μL silver nanoparticle sample was dropped on carbon-coated copper grids (PELCO, USA), air-dried, and then examined with TEM.

2. Preparation of MNPs

MNPs were prepared according to the reported procedure with slight modification.[1] $FeCl_3 \cdot 6H_2O$ (1.35 g, 5 mmol) was dissolved in ethylene glycol (40 mL) to form a clear solution, followed by the addition of NaAc (3.6 g) and polyethylene glycol (1.0 g). The mixture was stirred vigorously for 30 min and then sealed in a teflonlined stainless-steel autoclave (50 mL capacity). The autoclave was put in an oven at 200 °C for 8 h, and allowed to cool to room temperature. The black products were washed several times with ethanol and stored in 5°C. TEM images of MNPs are shown in Figure S1a and Figure S1b.

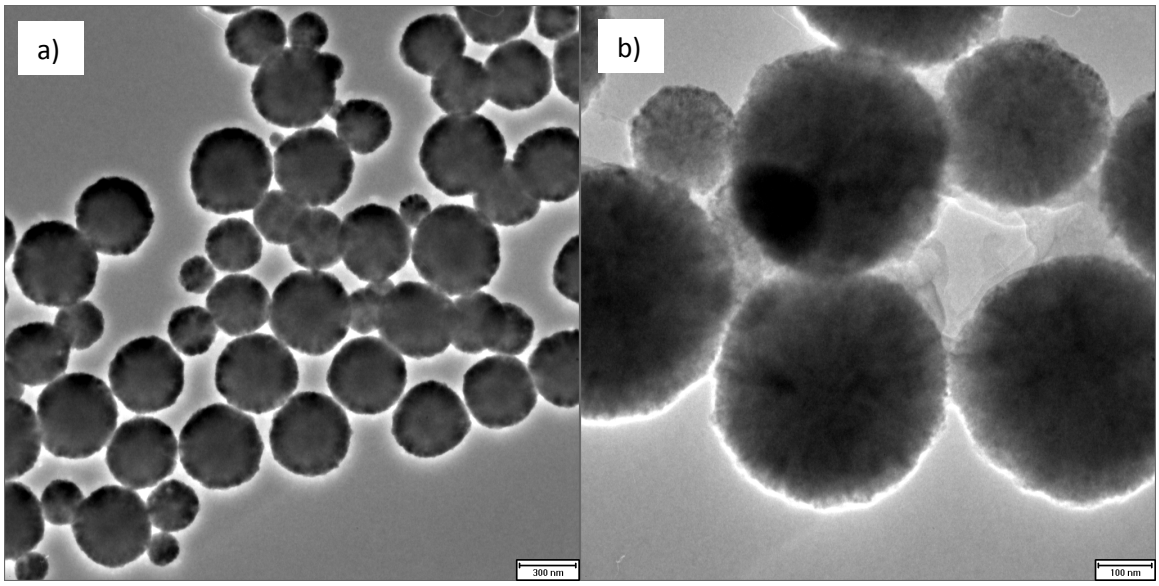

Figure S 1 TEM images of MNPs presented with 300-nm (a) and 100-nm (b) scale bar. The diameter of MNP is (186 ± 8) nm (n = 50).

3. Synthesis of MNP-Ag

MNP-Ag were prepared according to the reported procedure with slight modification.[2] One milliliter of MNPs were washed with water three times, and dissolved in 1 mL of 0.1 M $AgNO_3$. Then 1 mL of 100 mM $NaBH_4$ was added to the solution, and the solution was sonicated for 10 min. The resulting nanoparticle solution was washed three times with water. TEM images of MNPs are shown in Figure S2a and Figure S2b.

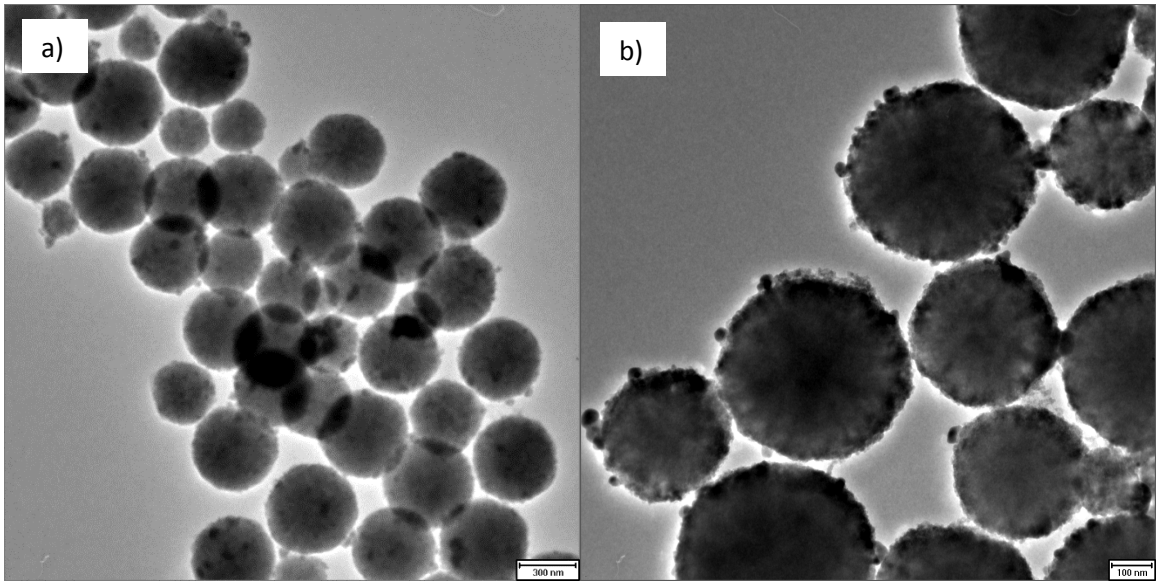

Figure S 2 TEM images of MNP-Ag presented with 300-nm (a) and 100-nm (b) scale bar.

4. Modification of MNP-Ag with DNA

Thiol-DNA functionalized MNP-Ag were prepared according to the literature with slight modification.[3] Specifically, 1000 μL of MNP-Ag were mixed with 100 μL of 10 μM TCEP treated HS-DNA (TCEP: DNA =100:1) with the concentration as indicated (DNA: MNP-Ag=400:1), then 500 μL of 100 mM citrate buffer (pH 3.0) was added. The solution was shaken in dark for 10 min, then separated by magnet and washed with purified water. The procedure was repeated three times to remove the DNA that was not attached to MNP-Ag. Then the MNP-Ag -DNA was redispersed in 1000 μL of 20 mM pH 7.5 phosphate buffer. One hundred milliliters of 10 mM mercaptoethanol were added to the suspension. After 1 h shaking, the suspension was separated by magnet and washing three times with purified water before redispersed in 1000 μL of 20 mM pH 7.5 phosphate buffer. For comparison, we have performed the same procedure with MNP without AgNP modification. The significant difference in fluorescence spectroscopy shows that the thiol-DNAs were specifically connected to the AgNP on MNPs, and the MNP without AgNP modification was not a good substrate for DNA modification.

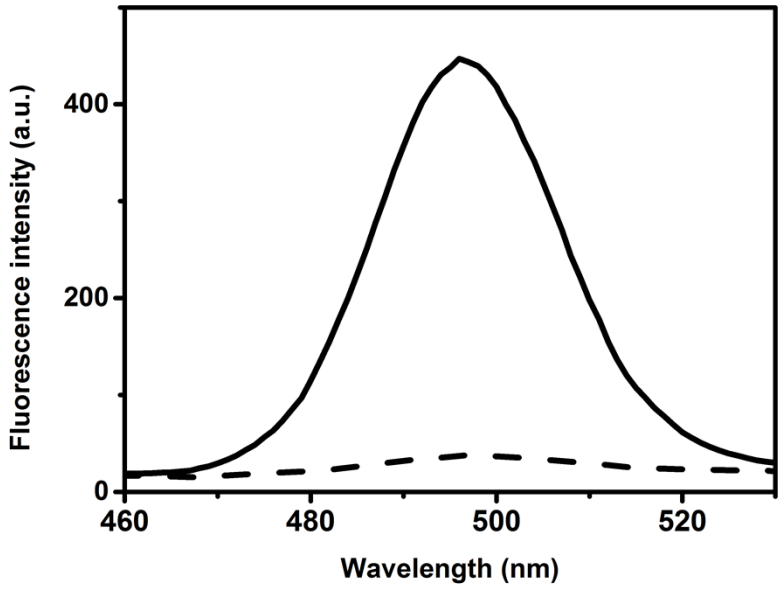

Figure S 3 Fluorescence spectra of FAM-labeled DNA modified MNP with (solid line) and without (dashed line) AgNP modification on the MNP surface.

5. Preparation of AgNPs

AgNPs were prepared according to the reported procedure with slight modification.[4] One hundred microliters of 0.1 M $AgNO_3$, 400 μL of 10 M NH_3 and 20 μL of 0.1 M NaOH were mixed with 20 mL of distilled water. Then 100 μL of 1 M glucose was added. After 30-min reaction, the AgNPs solution was

centrifuged for 5 min at 8,000 rpm. The AgNPs were washed three times by 4 mL of distilled water followed by 5 min sonication and centrifugation. The concentration of the AgNPs was estimated to be 0.5 nM. The as prepared AgNPs presented a size distribution of 89±2 nm (*n*=50), as characterized in our previous publication[5].

6. Immobilization of DNA on AgNPs

Thiol-DNA functionalized AgNPs were prepared according to the literature with slight modification.[3] Specifically, 100 μL of AgNPs were mixed with 100 μL of TCEP treated HS-DNA (TCEP: DNA =100:1) of the concentration as indicated (DNA: AgNPs=400:1), then 10 μL of 1 M citrate buffer (pH 3.0) was added. The solution was placed in dark for 10 min, and then centrifuged for 20 min at 13,000 rpm. The sediment was washed with purified water and centrifuged three times to remove the DNA that was not attached to AgNPs. Then the AgNP-DNA was redispersed in 100 μL of 20 mM pH 7.5 phosphate buffer.

7. Detection of target DNA

Fifty microliters of capture DNA connected to the MNP-Ag (indicate as MNP-capture), 50 μL of binding DNA and MEF DNA modified AgNP (indicate as reporter), 200 μL of 1×PBS, and 100 μL target DNA was mixed. The solution was heated to 70 ^{o}C and slowly cooled to room temperature after 10 min.

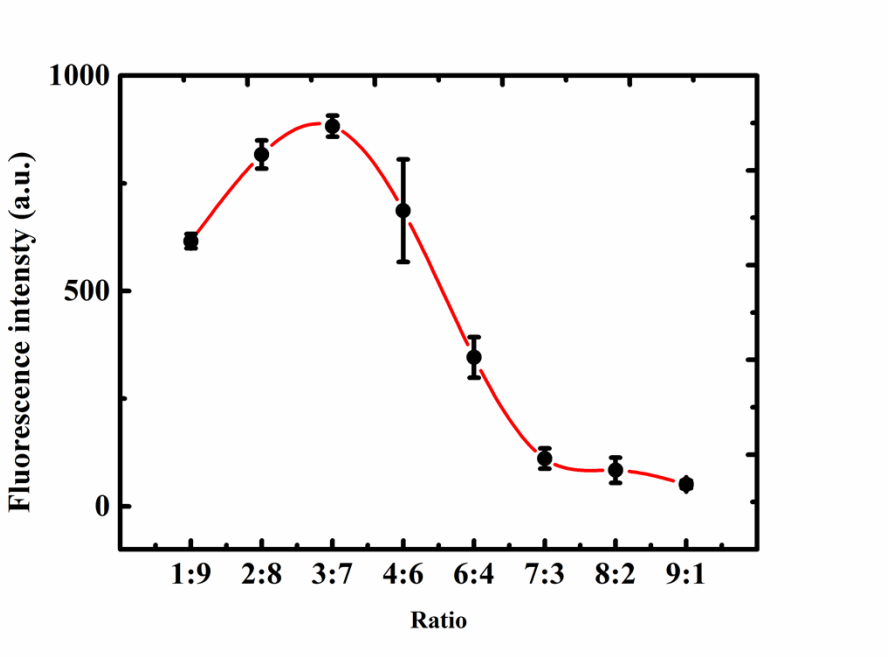

Figure S 4 The effect of binding DNA to barcode DNA ratio. Concentration of the target DNA was 500 pM.

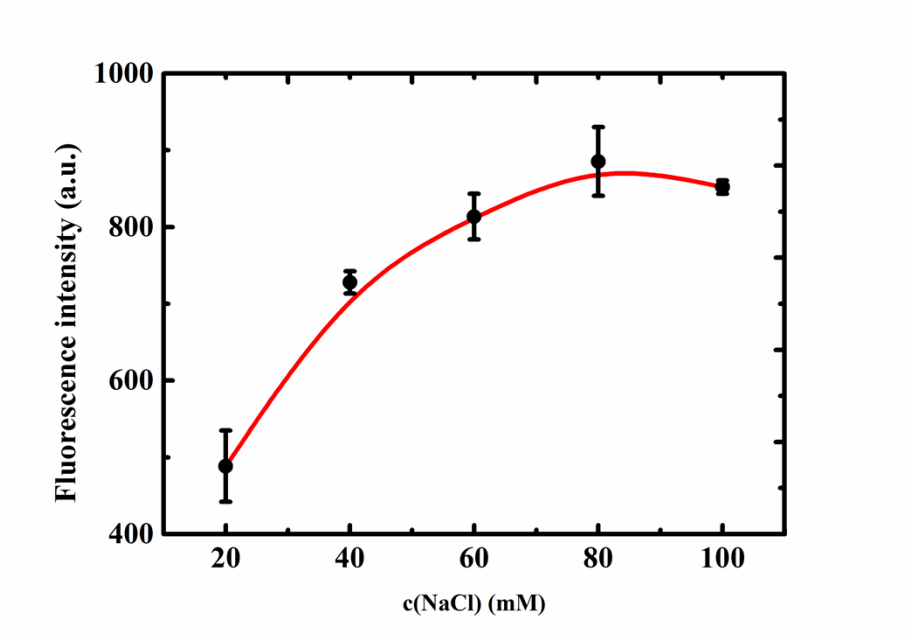

Figure S 5 The dependency of NaCl concentration. Concentration of target DNA was 500 pM.

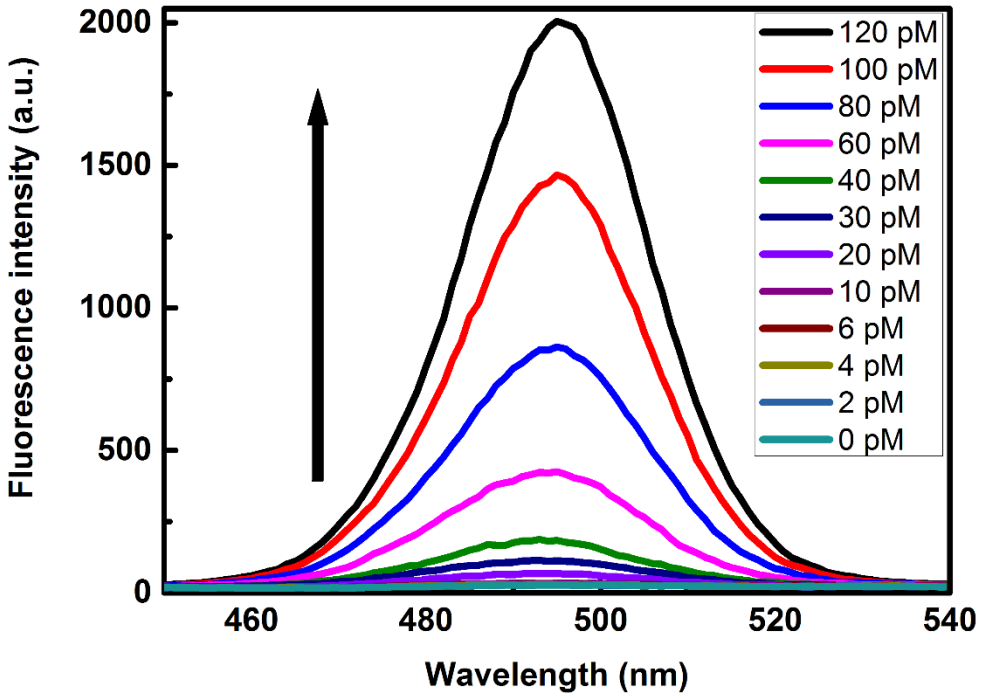

Figure S 6 The fluorescence spectra of the assay system as a function of the amount of target DNA.

References:

1. H. Deng, X. Li, Q. Peng, X. Wang, J. Chen and Y. Li, *Angew. Chem. Int. Ed. Engl.*, 2005, **44**, 2782-2785.
2. J. Baniukevic, I. H. Boyaci, B. A. Goktug, U. Tamer, A. Ramanavicius and A. Ramanaviciene, *Biosens. Bioelectron.*, 2013, **43**, 281-288.
3. X. Zhang, M. R. Servos and J. W. Liu, *J. Am. Chem. Soc.*, 2012, **134**, 7266-7269.
4. L. Kvitek, R. Prucek, A. Panacek, R. Novotny, J. Hrbac and R. Zboril, *J. Mater. Chem.*, 2005, **15**, 1099-1105.
5. Z. Zhou, H. Huang, Y. Chen, F. Liu, C. Z. Huang and N. Li, *Biosens. Bioelectron.*, 2014, **52**, 367-373.