A triple-amplification colorimetric assay for antibiotics based on magnetic aptamer-enzyme co-immobilized platinum nanoprobes and exonuclease-assisted target recycling

Yangbao Miao[a], Ning Gan*[,a], Hong-Xia Ren[c], Tianhua Li[a], Yuting Cao[a], Futao Hu[a], Zhongdan Yan[a], Yinji Chen[b]

[a] Faculty of Material Science and Chemical Engineering, Ningbo University, Ningbo, China, 315211

[b] Faculty of Food Science and Engineering, Nanjing University of Finance and Economics, Nanjing, China, 210000

[c] Key Laboratory of Asymmetric Synthesis and Chirotechnology of Sichuan Province, Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences, Chengdu, China, 610041

Supplementary Information

reagents and chemicals

The oligonucleotides used in this paper are as the following sequences: thiolated 40 mer Apt [1], 5'$(CH_2)_6$-ACT TCA GTG AGT TGT CCC ACG GTC GGC GAG TCG GTG GTAG; thiolated 40- mer complementary DNA (cDNA), 5'SH-(CH2)6-CTA CCA CCG ACT CGC CGA CCG TGG GAC AAC TCA CTG AAGT were purchased from Shanghai Sangon Biological Engineering Co., Ltd (Shanghai, China). Exo I and NEBuffer were got from New England Biolabs Ltd. (Beijing, China). CAP, streptomyces erythreus (ME), oxytetracycline (OTC), chlortetracycline (CTC), kanamycin (Kana) and gentamicin sulfate (GS) were purchased from Sigma (Milan, Italy). Hydrogen tetrachloroaurate(III) tetrahydrate $HAuCl_4$, Ferric chloride (FeCl3•6H2O), sodium acetate anhydrous (NaAc), hydrogen peroxide (H_2O_2, 30%) and 3,3',5,5'-tetramethylbenzidine (TMB) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The ds-DNA antibody ELISA Kit was purchased from Takara Biotechnology Co. Ltd. (Dalian, China). Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) was purchased from Sigma-Aldrich. All other reagents were analytical grade and were used without further purification. Double-distilled water was used throughout the study.

Apparatus

The transmission electron microscopic (TEM) image was obtained from a H600 transmission electron microscope (Hitachi, Japan). Scanning electron micrographs (SEM) were obtained from a S3400N scanning electron microscope (Hitachi, Japan). The UV-vis spectra were recorded by a UV-1800 spectrophotometer (Shimazu Co., Japan). Dynamic Light Scattering was carried out by the Malvern zetasizer Nano ZS90, Malvern instruments Ltd., UK with a 50 mV laser. Optimization of experimental condition.

The Gel electrophoresis experiment was employed to explain the feasibility of the signal amplification by Exo I.

2.5μL of the supernatant (The supernatant obtained by 100 ng mL^{-1} CAP 1mg mL^{-1} and probe reacting with 30 min.) and precipitate were loaded into a well of agarose gel electrophoresis. Electrophoresis was performed with vertical slab gel electrophoresis using 3.5g agarose and 10% TBE buffer (0.045mol/L Tris, 0.001mol/L EDTA, pH=8.0). The gel separation was done at 120 Volts. After completion of electrophoresis gels were developed by Coomassie staining.

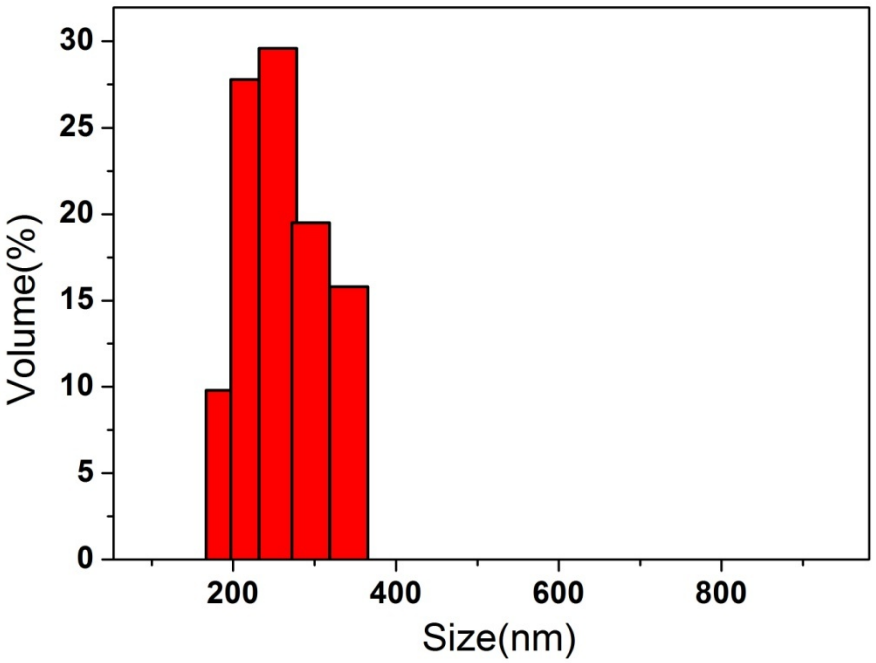

Fig.S1 Dynamic light scattering (DLS) measurement revealed that the Au MNPs has a mean hydrodynamic diameter of 242.25 nm.

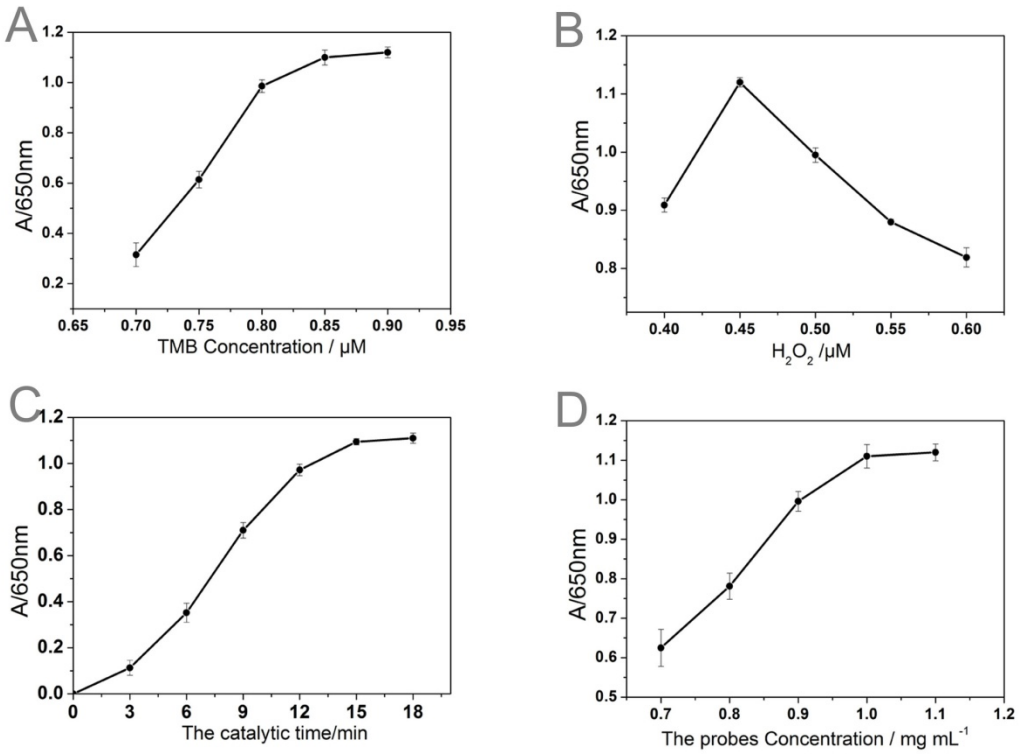

Fig.S2UV-vis absorption spectra of the proposed colorimetric aptasensor in the presence of different concentration of TMB (A), H_2O_2(B), magnetic compostie probes (D) and the optimal catalytic time of HRP in the supernatant the range from 0 min to 18 min(C).

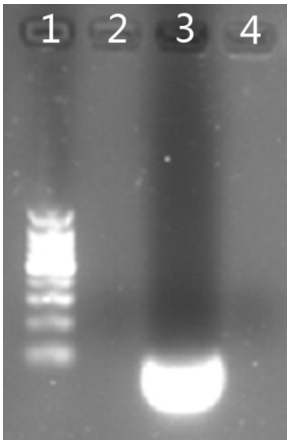

Fig.S3 SYBY-stained agarose gel electrophoresis demonstrates the signal amplification. lane 1, 50 bp marker; lane 2 the blank; lane 3, the supernatant after the reaction between 100 ng mL^{-1}CAP and 1 mg $mL^{-1}$the probe; lanes 4, the supernatant with the reaction between 100 ng mL^{-1}CAP, 20 U Exo I and 1 mg $mL^{-1}$the probe. Incubate time was 30 min.

References

1 J. Mehta, B. Van Dorst, E. Rouah-Martin, W. Herrebout, M. Scippo,R. Blust, J. Robbens, Journal of Biotechnology, 2011, **155**, 361–369.