Electronic Supplementary Information

One-pot synthesized DNA-templated Ag/Pt bimetallic nanoclusters as peroxidase mimics for colorimetric detection of thrombin

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EXPERIMENTAL SECTION

Reagents and Apparatus

Oligonucleotides in this study were synthesized by Shanghai Sangon Biotechnology Co., Ltd., which were purified by HPLC and confirmed by mass spectrometry. Table S1 displays the sequences of the used oligonucleotides. Each oligonucleotide was heated to 90 °C for 5 min and slowly cooled down to room temperature before use. High-binding polystyrene 96-well microplates were obtained from Greiner Bio-One (Frickenhausen, Germany). Silver nitrate ($AgNO_3$), human thrombin, lysozyme, bovine thrombin, human serum albumin (HSA), and human immunoglobulin (lgG) were obtained from Sigma-Aldrich Chemical Co., Ltd.. Potassium tetrachloroplatinate (II) (K_2PtCl_4) was purchased from J&K chemical Co., Ltd.. Hydrogen peroxide (H_2O_2) was purchased from Sinopharm Chemical Reagent Co., Ltd.. 3,3,5,5-Tetramethylbenzidine dihydrochloride (TMB) and sodium borohydride ($NaBH_4$) were purchased from Shanghai Sangon Biotechnology Co., Ltd.. Ultrapure water purified with a Millipore water purification system (18 MΩ resistivity) was used in all runs. Other chemicals were all of analytical grade and used as received.

Ultraviolet-visible light (UV-vis) absorption spectra were measured using a Tecan Infinite M200 PRO plate reader (Tecan, Austria) at room temperature. Fluorescence spectra were collected using a Hitachi Model F-4600 Fluorometer (Hitachi, Japan). Transmission electron microscopy (TEM) images, together with energy-dispersive X-ray (EDX) spectroscopies, were recorded on a Tecnai G2 20-TWIN field emission

scanning electron microscope (FEI, USA). The chemical composition was tested by X-ray photoelectron spectrometer model ESCALAB 250 (Thermo Electron Corporation, USA).

Table 1 Sequences of the used oligonucleotides

Name	**Sequence (5′-3′)**
template DNA	CCCCCTAACTCCCCC
random DNA	GCGACATGGTAATGG
Apt15	***GGTTGGTGTGGTTGG***
template-Apt15	CCCCCTAACTCCCCCTTTTTTTTTT***GGTTGGTGTGGTTGG***
botin-Apt29	botin-TTTTTTTTTT**AGTCCGTGGTAGGGCAGGTTGGGGTGACT**

The italic bold letters of Apt15 and template-Apt15 represent 15-mer thrombin aptamer. The underlined bold letters of botin-Apt29 are the sequence of 29-mer thrombin aptamer.

Synthesis of DNA-Ag/Pt Bimetallic NCs

Upon optimizing synthesis conditions, $AgNO_3$ solution (150 μM, 5 μL) and K_2PtCl_4 solution (125 μM, 12 μL) were added to the template DNA solution (2 μM, 30 μL) in 10 mM phosphate buffer (pH 7.0). After incubation for 30 min in the dark, a freshly prepared $NaBH_4$ (5 mM, 3 μL) solution was added under vigorous shaking in order to initiate the reduction reaction. The mixture allowed to react at 37 °C for up to 3 h. The concentration of the resulted DNA-Ag/Pt NCs was denoted as the concentration of DNA used in the clusters formation. To obtain DNA-Ag/Pt NCs with high catalytic activity, $AgNO_3$ and K_2PtCl_4 concentrations, as well as the preparation time, have been optimized in our synthesis.

Catalytic Activity Evaluation of DNA-Ag/Pt Bimetallic NCs

In a typical colorimetric reaction, 10 μL of 2 μM DNA-Ag/Pt NCs and 20 μL of 500 mM H_2O_2 were added into 130 μL of 200 mM acetate buffer (pH 4.0). A freshly prepared TMB stock solution (4 mM) was then added to the above solution to reach a final concentration of 800 μM. The enzymatic activity was followed by monitoring product formation for 10 minutes at 652 nm with a UV-vis spectrophotometer.

Fabrication of Colorimetric Aptasensor Based on DNA-Ag/Pt Bimetallic NCs

Prior to the assay, aptamer-functionalized Ag/Pt NCs were prepared using a ssDNA (template-Apt15 in Table S1) containing template DNA and 15-mer thrombin aptamer. The detailed procedure was described as follows: First, 96-well microtiter plates were coated with 50 μL per well of streptavidin at a concentration of 200 μg mL^{-1} in 50 mM sodium carbonate buffer (pH 9.6) for 2 h at 37 °C and then 12 h at 4 °C. In order to suppress the nonspecific adsorption to the plates, the microtiter plates should be blocked with 200 μL per well of blocking buffer (0.1% BSA) for 2 h at 37 °C. After that, biotinylated 29-mer aptamer (botin-Apt29 in Table S1) was immobilized on the streptavidin-coated microtiter plates through biotin-streptavidin interaction for the incubation of 1 h at 37 °C. Washing procedure should be performed after each modification steps. Second, 50 μL of human thrombin standards or 10-fold diluted human serum samples spiked with various concentrations of human thrombin were added and incubated for 1 h at 37 °C under shaking. Then, after washing, we added 50 μL per well of aptamer-functionalized Ag/Pt NCs (500 nM) in 100 mM phosphate buffer (pH 7.4) containing 1 mM $MgCl_2$ and incubation continued for 1 h at 37 °C, followed by washing again. Third, the enzymatic reaction was initiated by adding 100

μL of substrate solution containing 50 mM H_2O_2 and 800 μM TMB in 200 mM acetate buffer (pH 4.0). After incubation for 10 min at 37 °C, the catalytic reaction was stopped by adding 50 μL of 2 M H_2SO_4 to each well. The UV-vis absorption spectra of color products were recorded at 452 nm.

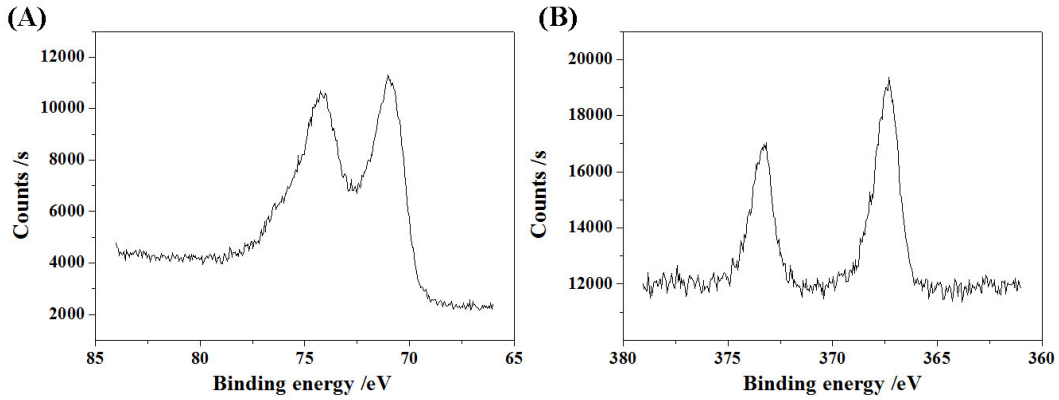

Fig. S1 XPS spectra for DNA-Ag/Pt NCs indicative of (A) Pt 4f and (B) Ag 3d.

Chemical investigation of DNA-Ag/Pt NCs was realized by X-ray spectroscopy (XPS) after drying the solution on a silicon wafer (Fig. S1). It can be observed that the Pt $4f_{7/2}$ peak appeared at about 71.09 eV, which could be attributed to the reduced platinum Pt(0).[1] The peak for Ag $3d_{5/2}$ in the DNA-Ag/Pt NCs occurred at 367.63 eV, revealing the existence of oxidized Ag species.[2]

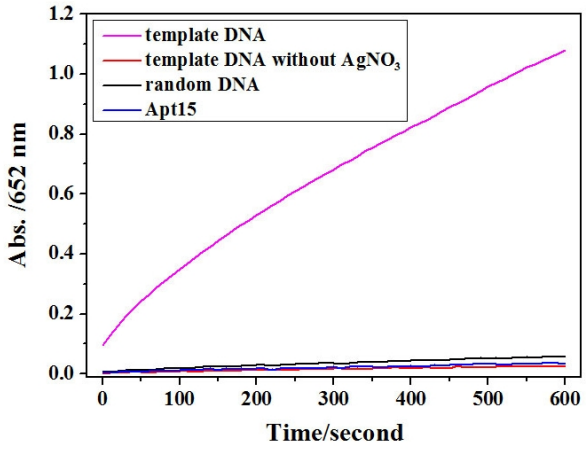

Fig. S2 Catalytic activity of Ag/Pt NCs in different synthesis conditions: (a) templated by template DNA; (b) templated by template DNA without $AgNO_3$; (c) templated by random DNA; (d) templated by Apt15. Experiments were performed in TMB-H_2O_2 system.

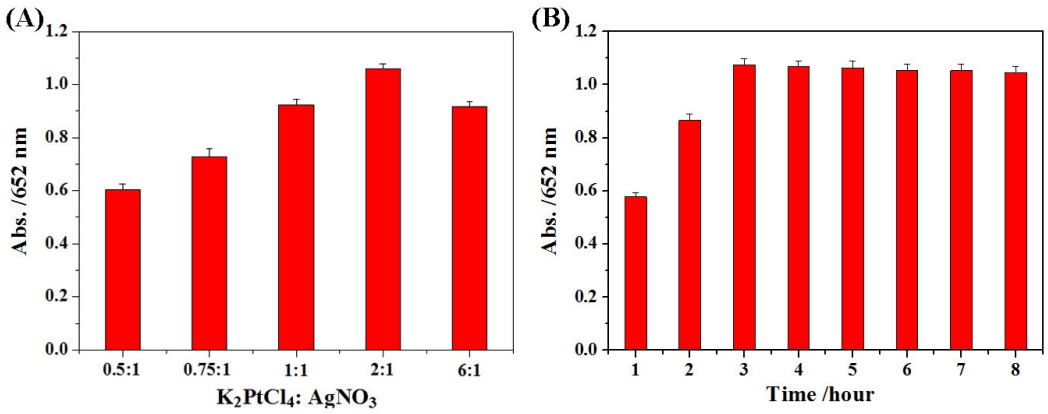

Fig. S3 Effect of (A) concentration ratio of K_2PtCl_4:$AgNO_3$ and (B) preparation time for the synthesis of DNA-Ag/Pt NCs on their peroxidase-like activities. Experiments were performed in 200 mM acetate buffer (pH 4.0) containing 100 nM DNA-Ag/Pt NCs, 50 mM H_2O_2 and 800 μM TMB. The above data shown here represented the means and standard deviations of three independent experiments.

We have carried out an experiment on the catalytic behavior of DNA-Ag/Pt NCs as a function of the K_2PtCl_4:$AgNO_3$ ratio used in the preparation process (Fig. S3A). The catalytic activity of DNA-Ag/Pt NCs increased with the increasing of K_2PtCl_4:$AgNO_3$ ratio, implying that more and more Pt NCs have been formed. DNA-Ag/Pt NCs obtained at 2:1 ratio of K_2PtCl_4 to $AgNO_3$ exhibited the maximum peroxidase-like catalytic activity. However, the further increase in K_2PtCl_4 concentration led to the reduced catalytic activity, which may result from the formation of larger Pt NPs. Thus the optimal molar ratio of K_2PtCl_4 to $AgNO_3$ was 2:1.

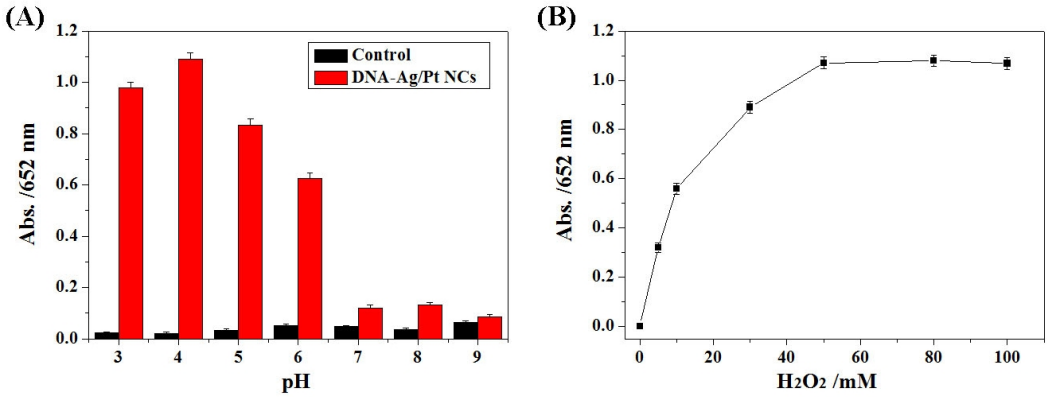

Fig. S4 Effect of (A) pH and (B) H_2O_2 concentration on the catalytic reaction of DNA-Ag/Pt NCs. The above data shown here represented the means and standard deviations of three independent experiments.

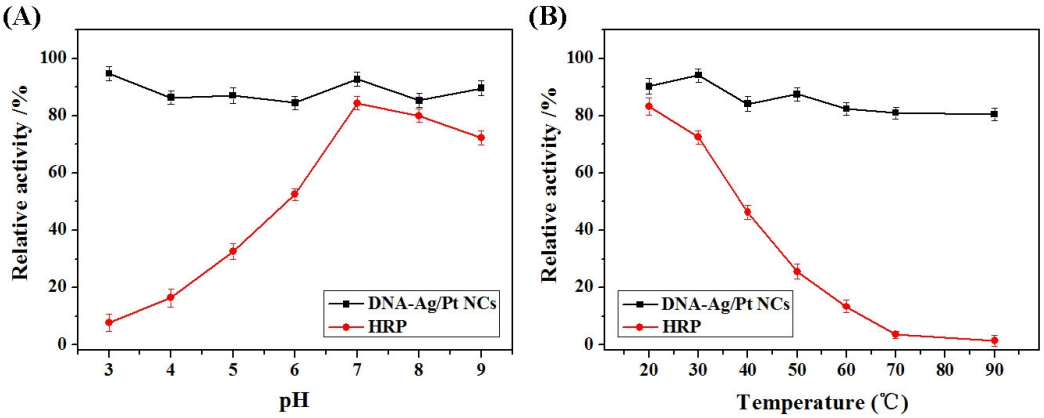

Fig. S5 Relative catalytic activity of DNA-Ag/Pt NCs and HRP against (A) pHs (3.0-9.0) and (B) temperatures (20-90 °C). Activity of DNA-Ag/Pt NCs was detected in 200 mM acetate buffer (pH 4.0) containing 100 nM DNA-Ag/Pt NCs, 50 mM H_2O_2 and 800 μM TMB. While the HRP activity was detected in 200 mM acetate buffer (pH 4.0) containing 100 pM HRP, 5 mM H_2O_2 and 800 μM TMB. The above data shown here represented the means and standard deviations of three independent experiments

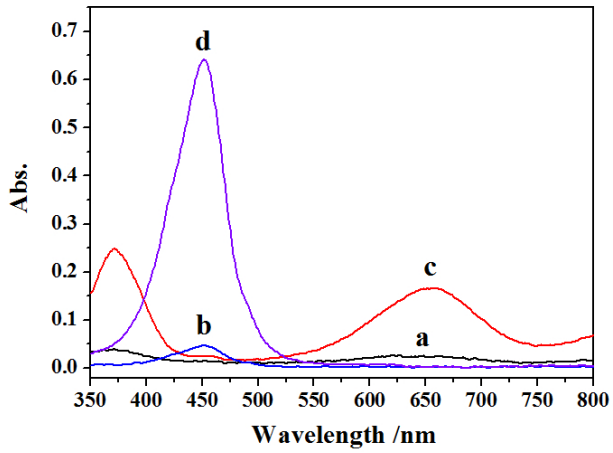

Fig. S6 UV−vis spectra of the TMB-H_2O_2 systems in the absence of DNA-Ag/Pt NCs before (a) and after (b) addition of H_2SO_4; in the presence of DNA-Ag/Pt NCs before (c) and after (d) addition of H_2SO_4.

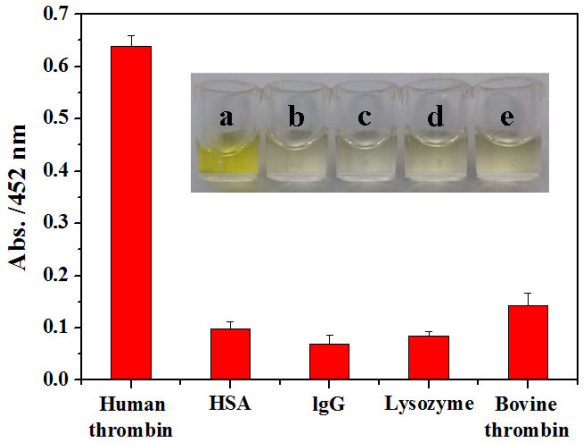

Fig. S7 The signal difference produced by the catalytic reaction in the proposed aptasensor towards (a) human thrombin, (b) HSA, (c) IgG, (d) lysozyme and (e) bovine thrombin (30 nM for each). Inset: photograph showing colorimetric responses for different proteins. The data shown here represent the average of three independent experiments.

Table S2 Recovery experiments of human thrombin spiked into diluted human serum samples

Samples[a]	Thrombin spiked (nM)	Thrombin detected (nM)	Recovery (%)	RSD (%)
1	10	9.65	96.5	5.4
2	20	22.04	110.2	4.3
3	50	53.9	107.8	4.6

[a] Each sample was analyzed in triplicate, and the results are the average value.

References

1. W. Zhang, J. Yang and X. Lu, *ACS Nano*, 2012, **6**, 7397-7405.

2. N. Bahadur, K. Jain, R. Pasricha and S. Chand, *Sens. Actuators B*, 2011, **159**, 112-120