Electronic Supplementary Information

Label-Free Detection of DNA by Combining Gated Mesoporous Silica and Catalytic Signal Amplification of Platinum Nanoparticles

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1. Experimental Section

1.1 Materials

Tetraethylorthosilicate (TEOS)，N-cetyltrimethylammonium bromide (CTAB)，3, 3, 5, 5-tetramethylbenzidine (TMB), 3-aminopropyl triethoxysilane (APTS) were purchased from Aldrich. H_2O_2, K_2PtCl_4, NaOH, $NaBH_4$, Ethanol, and Hydrochloric acid (HCl, 37%) were obtained from Shanghai Chemical Reagent Corporation. All chemicals were used as received. Oligonucleotides used in this study were synthesized and purified by Shanghai Shenyou Biotechnology Company. Water used in this experiment was purified by distillation of deionized water.

1.2 Synthesis of Pt nanoparticle (NP)

The synthesis of Pt nanoparticles was carried out by following the reported method with a modification. 5 mL of aqueous 10 mM K_2PtCl_4 and 12.5 mL of 400 mM CTAB were mixed with 29.5 mL of water in a 100-mL round bottom flask at room temperature. The mixture was stirred at room temperature for 10 min and was heated at 50 °C for 10 min. To the clear solution, 4 mL of 500 mM ice-cooled $NaBH_4$ solution was injected through the septum using a syringe. The gas evolved inside the flask was released by inserting a needle through the septum for 20 min. The needle was then removed and the solution was kept at 50 °C for 15 h. The product was centrifuged at 15000 rpm for 20 min, and the supernatant solution was separated. The Pt NP colloids were collected and re-dispersed in 5 mL of water by sonication for further use.

If we assume that the obtained Pt NP is sphere, the molarity of Pt NPs solution can be estimated as follows:

The number of the Pt atoms in one Pt nanoparticle with the size of 10 nm can be derived,

$$V_{\text{Pt NP}} = \frac{4}{3}\pi \times r^3$$

$$N_{\text{Pt/NP}} = \frac{\rho_{\text{Pt}} \times V_{\text{Pt NP}}}{M_{\text{w,Pt}}} \times N_A$$

It is known: r: the average radius of the Pt NP, 5 nm; $V_{\text{Pt NP}}$: the volume of one Pt NP; ρ_{Pt}: the density of Pt, 21.45g.cm^{-3}; $M_{w,\,Pt}$: the molar mass of Pt, 195.1 g.mol^{-1}; N_A: Avogadro's number; Therefore, $N_{\text{Pt/core}}=3\times10^4$.

And the molarity of Pt NPs solution can be derived by

$$M_{\text{Pt NPs}} = \frac{C_{\text{Pt}}}{N_{\text{Pt/NP}}}$$

$M_{\text{Pt NPs}}$: the molarity of Pt NPs solution; C_{Pt}: the molar concentration of Pt species;

So for the 0.01 M of Pt species solution, $M_{\text{Pt NPs}}$ is about 3.3×10^{-7} M

1.3 Synthesis of Pt@Mesoporous SiO_2 nanoparticle (Pt@mSiO_2 NPs)

The Pt@mSiO_2 core-shell NPs were prepared by polymerizing the silica layer around the

surface of Pt NPs using a sol-gel process according to the previous report with a modification.[1] The Pt NP colloid (1.67×10^{-9} mol) dispersed in 5 ml of water was firstly added to 30 mL of water. Then, 0.40 mL of NaOH solution (0.1 M) was added to the aqueous Pt colloid solution with stirring to adjust the pH of the solution to 10-11. To this basic solution, a controlled amount of 10 vol% TEOS diluted with methanol, which is determined by the thickness of silica shell (for instance, 0.8 mL), was added to initiate the silica polymerization. After the reaction, the resulting Pt@$mSiO_2$ NPs were washed by ethanol two times. Before the next step, the Pt@$mSiO_2$ NPs were dispersed in ethanol with the concentration of 3.3×10^{-7} M.

1.4 Surface modification of Pt@$mSiO_2$ NPs with APTS

The surface modification of Pt@$mSiO_2$ NPs with APTS was performed in ethanol solution at room temperature. Typically, to 10 mL of the ethanol solution with 3.3×10^{-7} M of Pt@$mSiO_2$ NPs, 40 μL of APTS was added. Then, the resulting reactant mixture was stirred for 15 h. Finally, the APTS-coated Pt@$mSiO_2$ NPs were separated from the mixture by the ultracentrifugation and washed with water for two times.

1.5 The removal of CTAB from Pt@$mSiO_2$ NPs

The removal of CTAB from the as-synthesized Pt@$mSiO_2$ was carried out by the extraction method. Typically, the removal of CTAB by NH_4NO_3/Methanol extraction was performed in 5 mL of methanol solution containing 30 mg of NH_4NO_3. Firstly, the obtained Pt@$mSiO_2$ NPs were added to the above solution. And the resulting mixture was then heated to reflux at 60 °C under stirring for 8 h. After the solvent extraction, Pt@$mSiO_2$ NPs were further washed by water for two times, and finally dispersed in water with the concentration of 3.3×10^{-7} M.

1.6 The conjugation of Pt@$mSiO_2$ NPs with single strand DNA probe (P1)

For the preparation and optimization of the gated material Pt@$mSiO_2$-P1, the effect of various reaction parameters, such as P1 concentration, the length of P1, and the incubation temperature, on the capping efficiency were studied in detail. In order to obtain positive charged Pt@$mSiO_2$, before the conjugation of Pt@$mSiO_2$ NPs with P1, the protonation of the amine group on Pt@$mSiO_2$'s surface was done by washing NPs with aqueous HCl (pH 5.2) for 2 times. In the general procedure, to 50 μL of H_2O/PBS buffer (6:4, pH 7.2) containing a controlled amount of oligonucleotide P1-30 (such as 4 μmol), 50 μL of aqueous Pt@$mSiO_2$ (6.7×10^{-8} M) were added. And the resulting mixture solution was continuously stirred for 30 min at 37 ºC. Then, the resultant Pt@$mSiO_2$-P1 was isolated by centrifugation and washed twice with 1 mL of PBS to eliminate the residual oligonucleotide P1-30. Finally, the obtained Pt@$mSiO_2$-P1 was dispersed in 100 μL of PBS. In other experiments, P1-50 (or P1-40, P1-20, P1-10) was used stead of P1-30, and the incubation temperature referred to 45 ºC, 50 ºC, 55 ºC or 60 ºC.

P1-50 5’ GCTTGATATTAGACTCATTCTTTCCTTGATTTTCTTCCTTTTGTTCACAT 3’
P1-40 5’ AGACTCATTCTTTCCTTGATTTTCTTCCTTTTGTTCACAT 3’
P1-30 5’ TCTTTCCTTGATTTTCTTCCTTTTGTTCAC 3’
P1-20 5’ CCTTGATTTTCTTCCTTTTG 3’
P1-10 5’ ATTTTCTTCC 3’

1.7 Evaluation of the capping efficiency by the catalytic oxidation of TMB

The capping efficiency of Pt@mSiO$_2$-P1 was studied by the catalytic oxidation of TMB. In the general procedure, 1 μL of aqueous Pt@mSiO$_2$-P1 obtained above was added to 0.5 mL of Acetate buffer (0.1 M, pH 4.7) containing 0.5 mM of TMB and 0.2 M of H_2O_2. After the reaction proceeded for a certain time, the resulting solutions were used for absorbance measurement at a wavelength of 652 nm on Thermo Scientific Evolution 60 S UV-Vis recording spectrophotometer.

1.8 Label-free DNA detection

To investigate the single base pair mismatch discrimination capability of Pt@mSiO$_2$-P1, four kinds of the single strand target DNA (T0, T1, T2, and T3) with different mismatched bases are designed. In the typical experiment, 10 μL of aqueous Pt@mSiO$_2$-P1 obtained above was firstly mixed with 8 μL of the hybridization buffer. Then, 2 μL of aqueous T0 (20 nM, or T1, T2, T3) was added and further incubated at 37 ºC for 30 min. After the hybridization, 1 μL of aqueous solution were taken from this suspension, and further added into 0.5 mL of Acetate buffer (0.1 M, pH 4.7) containing 0.5 mM of TMB and 0.2 M of H_2O_2. Following this step, the absorbance at a wavelength of 652 nm was measured.

Similarly, the sensitivity limit of Pt@mSiO$_2$-P1 was measured by the incubation with various concentration of T0.

T0 (complementary oligonucleotide) 5’ AAC AAA AGG AAG AAA ATC AAG GAA 3’
T1 (single-base mismatch sequence) 5’ AAC AAA AGG AAT AAA ATC AAG GAA 3’
T2 (two-base mismatch sequence) 5’ AAC AAA AGG AATGAA ATC AAG GAA 3’
T3 (three-base mismatch sequence) 5’ AAC AAA AGG ACTGAA ATC AAG GAA 3’

2. Characterization

2.1 Transmission electron microscope (TEM)

Sample for TEM and ED was prepared by placing a drop of the colloidal dispersion of Pt NPs or Pt@mSiO$_2$ NPs onto a carbon-coated copper grid followed by naturally evaporating the solvent. The electron microscope was a JEM-2100.

2.2 FT-IR Spectroscopy

FT-IR Spectra were recorded on a Perkin-Elmer FT-IR/Raman 2000 instrument in the

transmission mode. Samples were prepared as KBr disks (by mixing samples with spectroscopic grade KBr) and analyzed in the spectral range of 4000-400 cm^{-1}.

2.3 UV-Visible absorption Spectroscopy

UV-Visible absorption Spectra were recorded on a thermo Scientific Evolution 60 S UV-Vis recording spectrophotometer, between 350 and 750 nm wavelengths. And the samples were measured in a 1-mm quartz cuvette using the corresponding pure solvent as a reference.

2.4 Zeta potential measurement

Zeta potential measurement was conducted in Zetasizer NanoS from Malvern Instruments. Before the measurement, the particles were directly dispersed in deionized water.

3. FT-IR characterization of $Pt@mSiO_2$

Fig.S1 gives the FT-IR adsorption spectra of $Pt@mSiO_2$ before and after the removal of CTAB, and that with the surface modification of APTS, respectively. As shown in Fig.S1, the large amount of surfactant CTAB in $Pt@mSiO_2$ gives the characteristic C–H vibration bands at 2850~2930 cm^{-1} and C-H deformation bands at 1460 cm^{-1} before extraction. After the removal of surfactant CTAB by solvent extraction procedure, those IR peaks from CTAB molecules decrease significantly, suggesting the efficient removal of CTAB molecule. When the surface of $Pt@mSiO_2$ was modified with APTS, the new peaks at 2900~2930 cm^{-1}, which should be associated with –C–NH_2 bond (stretch vibration) and C–H bond (stretch vibration) in APTS, was observed. Taking all the above results together, it can be concluded that APTS coated $Pt@mSiO_2$ by was obtained.

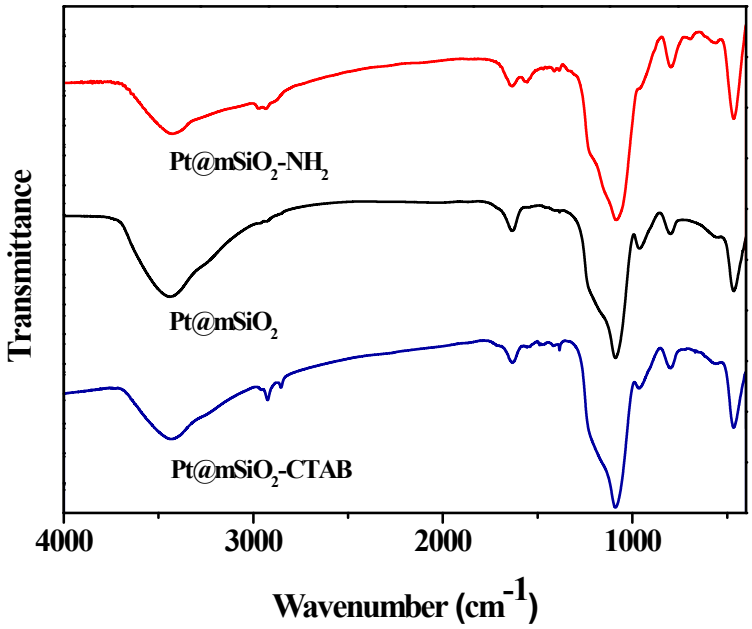


Fig. S1 FT-IR spectra of $Pt@mSiO_2$-CTAB (before the removal of CTAB), $Pt@mSiO_2$ (after the removal of CTAB), and $Pt@mSiO_2$ -NH_2 (modification with APTS)

4. Zeta potential measurement

In order to further track the evolvement of linked groups on the surface of $Pt@mSiO_2$, the zeta potential characterization was also conducted. After the removal of CTAB surfactant, the zeta potential of $Pt@mSiO_2$ change from -2 mV to -18 mV, showing the existence of a lot of silanol OH groups. Meanwhile, when being functionalized with APTES, the resulting $Pt@mSiO_2$ presents a positive zeta potential of around +20 mV. After the attachment of P1, its potential decreases to around -24.0 mV, indicating the existence of some oligonucleotides on the surface of APTS-modified $Pt@mSiO_2$.

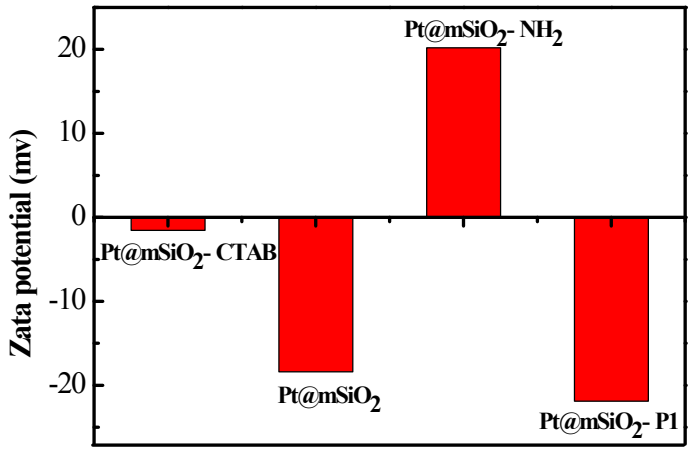


Fig. S2 Zeta potential of $Pt@mSiO_2$-CTAB, $Pt@mSiO_2$, $Pt@mSiO_2$-NH_2, and $Pt@mSiO_2$ capped by P1.

5. Effect of pH, TMB concentration, H_2O_2 concentration, and Pt@ $mSiO_2$ concentration on TMB-H_2O_2 colorimetric system

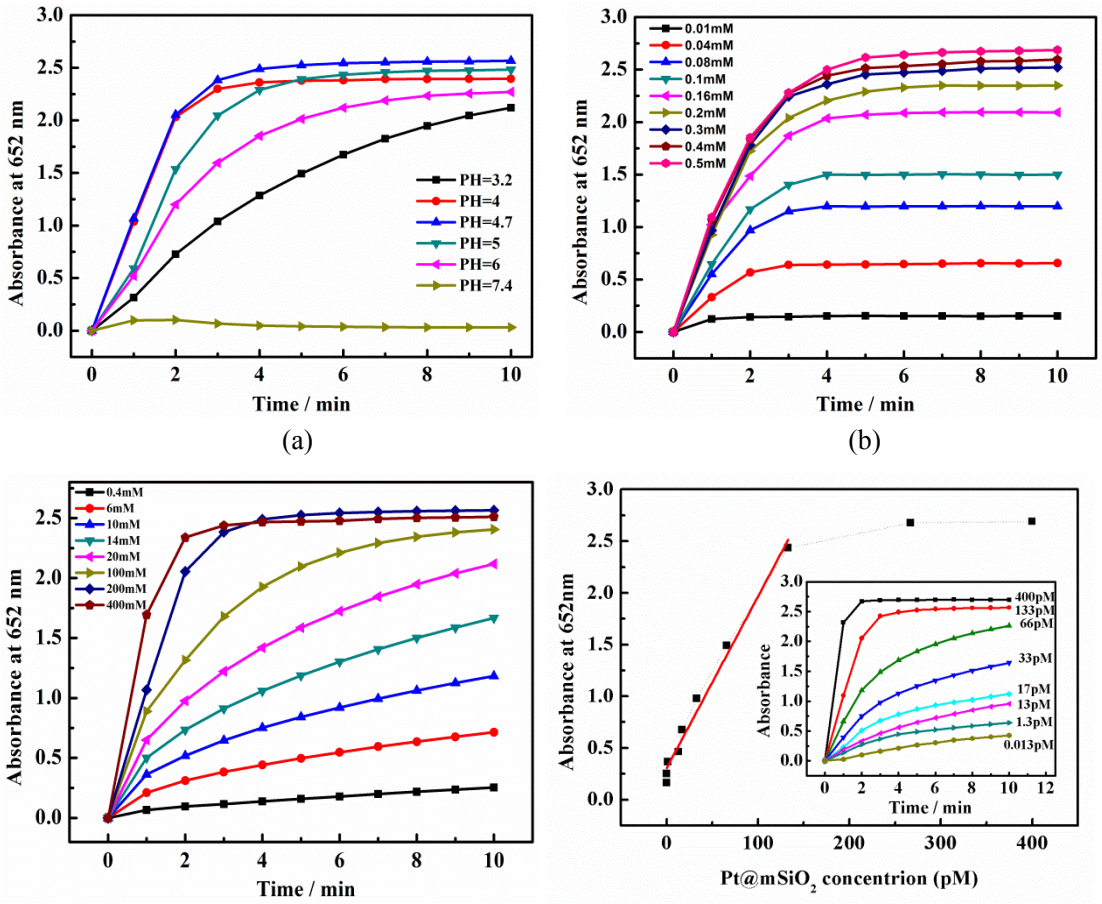


Fig. S3 Time-dependent absorbance changes at 652 nm of TMB reactant under the different reaction conditions: various pH (a), various TMB concentration (b), and various H_2O_2 concentration (c); (d) Relationship between absorbance obtained at 3 min and the concentration of Pt@$mSiO_2$ NPs. The straight line is a linear regression between absorbance and Pt@$mSiO_2$ NPs concentration ranging from 0 to 0.13 nM. The inset is the absorbance evolution over whole 10 min incubation for different Pt@$mSiO_2$ NPs concentrations.

6. Steady state kinetic assay

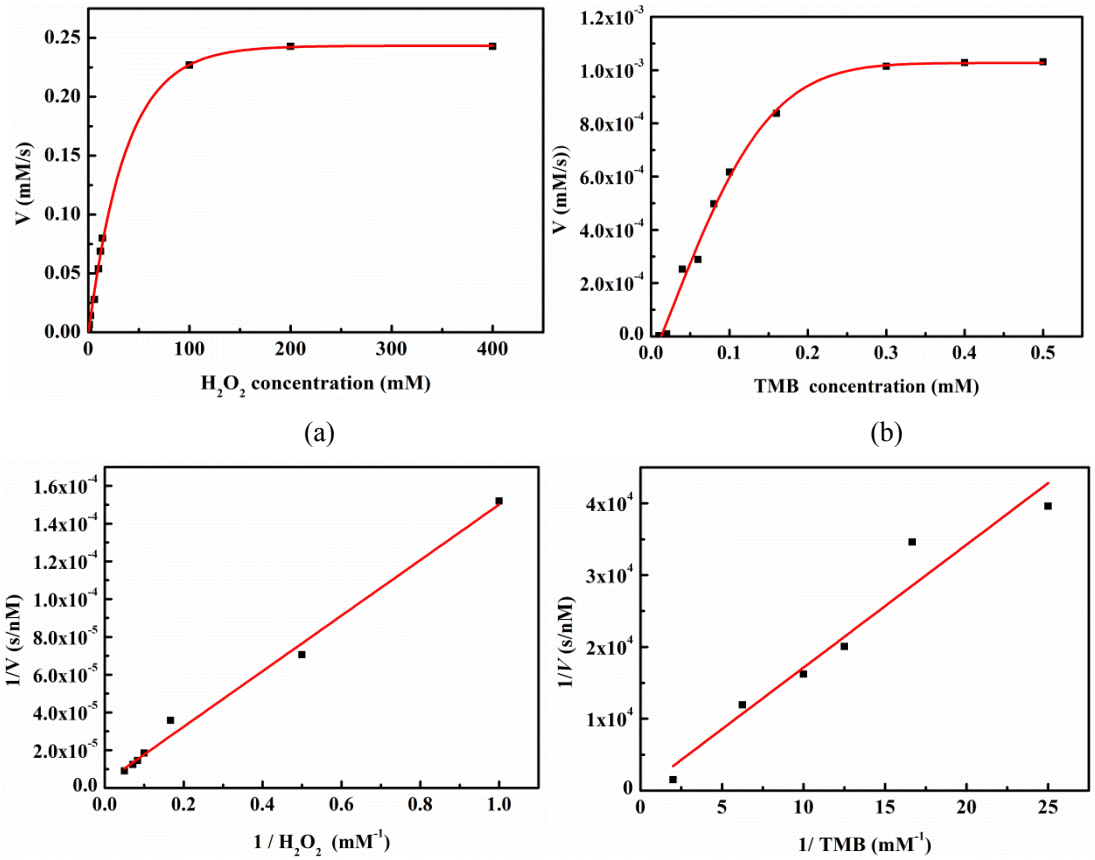
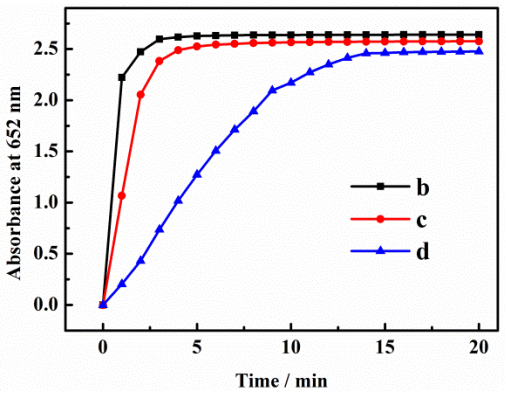


Fig. S4 Steady-state kinetic assay and catalytic characteristics of the Pt@mSiO$_2$ NPs toward various components: 0.5 mM TMB and different-concentration H_2O_2 (a, c); 0.2 M H_2O_2 and different-concentration TMB (b, d); The average rate (V) of the reaction was measured using 0.13 nM Pt@mSiO$_2$ NPs in 0.5 mL of Acetate buffer (0.1 M, pH 4.7) at room temperature.

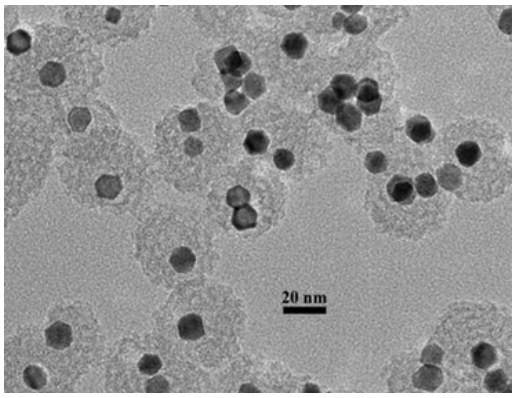
(H_2O_2 $y=1.5\times10^{-4}\times x+3.5\times10^{-6}$ ($K_m=2.35\times10^{-8}$ mM $V_{max}=$ 0.29 mM/s)

(TMB $y=1.5\times10^{-4}\times x+3.8\times10^{-4}$ ($K_m=2.55\times10^{-6}$ mM $V_{max}=$ 2.6×10^{-3}mM/s)

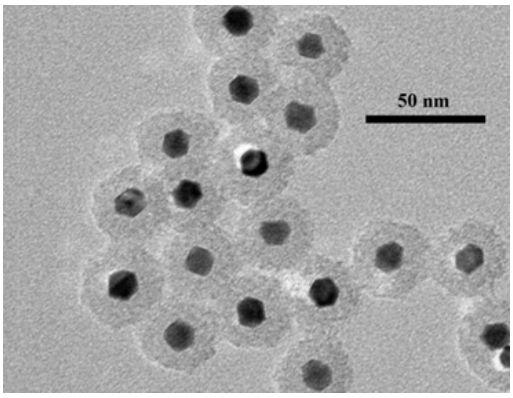
7. Effect of the thickness of silica shell on the catalytic behavior of Pt@ $mSiO_2$



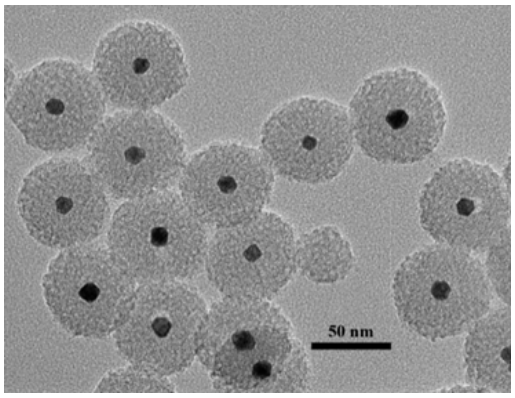
(a)



(b)



(c)



(d)

Fig. S5 (a) Time-dependent absorbance changes at 652 nm of TMB reactant catalyzed by Pt@ $mSiO_2$ NPs with different thickness of silica shell (for b, c, and d, their corresponding morphology of NPs were shown in TEM image (b), (c) and (d), respectively.); b, c, and d NPs were separately obtained by the addition of lower, medium and higher concentrations of TEOS during the preparation of Pt@ $mSiO_2$.

8. Effect of P1 concentration, the length of P1, and the incubation temperature on the capping efficiency

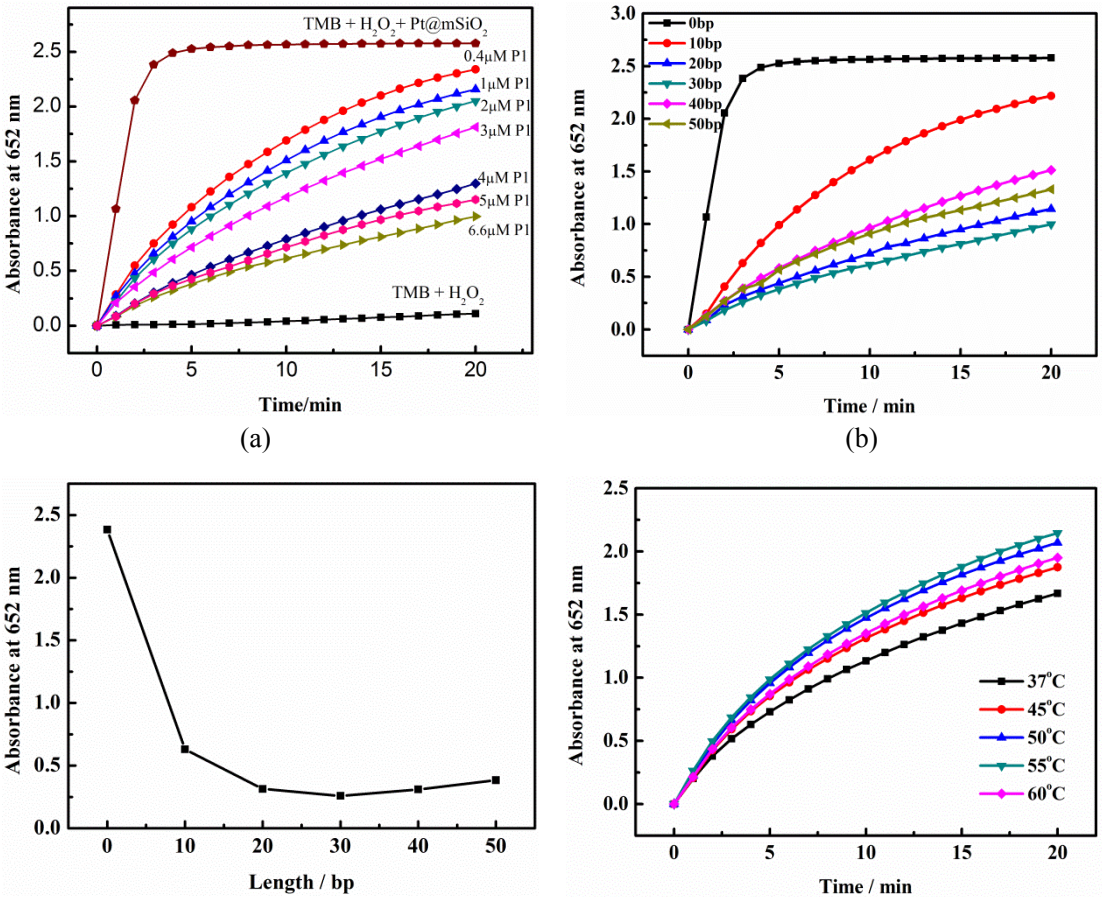


Fig.S6 Effect of P1 concentration (a), the length of P1(b, c), and the incubation temperature (d) on the capping efficiency.

References

1. S. H. Joo, J. Y. Park, C. K. Tsung, Y. Yamada, P. D. Yang and G. A. Somorjai, *Nat. Mater.*, 2009, **8**, 126–131.