Supporting Information

Highly efficient colorimetric detection of ATP utilizing split aptamer target binding strategy and superior catalytic activity of graphene oxide-platinum/gold nanoparticles

Siqi Zhang a, Kun Wang a,b, Jiali Li a, Zhenyu Li a, Ting Sun a*

a College of Sciences, Northeastern University, Shenyang, 110819, China
b Department of Chemistry and Environmental Engineering, Changchun University of Science and Technology, Changchun, China

*Corresponding author

E-mail: sun1th@163.com
Tel.: +86-024-83684786;

Preparation of Aptamer-1 Modified Magnetic Beads.

The Fe₃O₄ nanoparticles with the size of 200 nm were synthesized according to solvothermal method¹. FeCl₃•6H₂O (0.54 g), Na acrylate (1.5 g), NaOAc (1.5 g), were dissolved in a mixture of ethylene glycol (20 mL) under magnetic stirring. The obtained mixture solution was transferred into a Teflon stainless-steel autoclave and heated at 200°C for 10 h. When the autoclave was cooled down to room temperature, the black products were washed four times with ethanol and ultrapure water, respectively. Then the magnetic Fe₃O₄ microspheres were dispersed in a mixture of
ethanol (80 mL) and ultrapure water (20 mL) under ultrasonication for 5 min. Ammonia (1 mL) and TEOS (200 μL) was added into the mixture solution under mechanical stirring for 12 h. After being separated, the product mixed with APTES (100 μL) and ammonia (2 mL) under mechanical stirring for another 12 h. Finally, the Fe₃O₄@SiO₂-NH₃ was magnetic separated three times and re-dispersed in 5 mL ethanol/H₂O solution. The diameter of the Fe₃O₄@SiO₂-NH₃ was about 200 nm evaluated by TEM measurements.

The aptamer-1 functionalized Fe₃O₄@SiO₂ was prepared as follows. Fe₃O₄@SiO₂-NH₃ in 100 μL ethanol/H₂O solution was first mixed with 20 μL Tween 20 (5% in ethanol solution) at room temperature. Then carboxyl modified aptamer-1 (final concentration of oligonucleotides 10 μM) in 400 μL 10 mM MES buffer with 200 mM EDC and 50 mM NHS was added into the Fe₃O₄@SiO₂ suspension. The mixture solution was mechanical stirred at 37 °C for 16 hours. To remove excess aptamer, these aptamer-functionalized Fe₃O₄@SiO₂ were magnetic separated and washed three times using 10 mM Tris-HCl buffer containing 5 mM MgCl₂ and 15 mM KCl (pH=8.0). The functionalized Fe₃O₄@SiO₂ nanoparticles were stored in the buffer solution.
2.5 Preparation of PDDA-Functionalized Graphene Oxide.

The graphene oxide was prepared from graphite powder according to the previous work. Then 80 mg of PVP was upon addition of 20 mL of 0.25 mg/mL obtained graphene oxide solution. After magnetic stirring 30 min, the dispersion was centrifuged separation three times and re-dispersed in 5 mL ultrapure water. The PDDA-functionalized graphene oxide was prepared by mixing 0.1 mL of 20 wt %
PDDA with 16.8 mL of 0.625 M KCl, followed by adding 4.2 mL of PVP-coated graphene oxide. After sonication for 90 min, the PDDA/GO dispersion solution was centrifuged separation three times and re-dispersed in 5 mL ultrapure water.

2.6 Preparation of Aptamer-2 Modified Graphene Oxide/Platinum/Gold Nanoparticle Hybrids.

The Platinum/gold nanoparticle hybrids were synthesized with citrate reduction according to previous work. A 100 mL sample of aqueous (0.9 mM) HAuCl₄ and K₂PtCl₆ (0.1 mM) was prepared in a 250 mL round-bottom flask. The solution was continuously heated to a boil while stirring, and 40 mM trisodium citrate solution (10 mL) was added. The boiling and stirring was continued for an additional 30 min after the final color changing from pale yellow to deep red.

1 mL PDDA/GO dispersion solution (1 mg/mL) was added into 10 mL of prepared PtAu NPs under magnetic stirring. Then the dispersion solution was sonicated for 10 min before overnight aging. The obtained product was washed three times and dispersed in 2 mL of water.

The aptamer-2 functionalized GO/PtAu NPs was prepared by mixing GO/PtAu NPs (2 mL) with thiol-aptamer (final concentration of oligonucleotides 10 μM) in PBS buffer (0.3 M NaCl, 0.2 PBS, pH=7) under continuous shaking for 16 hours. To remove excess thiol-aptamer, these aptamer-2 functionalized GO/PtAu NPs were centrifuging for 45 min at 13000 rpm three times using 10 mM Tris-HCl buffer containing 5 mM MgCl₂ and 15 mM KCl (pH=8.0). The functionalized GO/PtAu NPs
nanoparticles were stored in the buffer solution. The synthesized nanomaterials were
shortly named as GO/PtAu\textsubscript{NP}/Aptamer-2.
As shown in Figure S1 (supporting information), the functionalized GO/PtAu NPs and magnetic bead were characterized by measuring the UV absorbance spectra after removing excess DNA. Except for the feature peaks of GO at 270 nm and PtAu at 536 nm, a new and higher peak at 260 nm was detected for the GO/PtAu NPs/aptamer-2 (curve c in Figure S1A), indicating the successful anchoring of aptamer-2 on the surface of GO/PtAu NPs. A similar peak at 260 nm was observed for aptamer-1-modified magnetic beads (curve b in Figure S1B).

The absorbance value of the functionalized nanoparticles was used to estimate the amount of aptamers after deducting the absorbance value of naked nanoparticles at 260 nm. According to oligonucleotide synthesis reports, the reciprocal of molar extinction coefficients for aptamer-1 and aptamer-2 are 7.0 and 3.9 nmol/OD, respectively. The amount of aptamer-1 on the magnetic beads was 10.94 nmol/mg.
while the amount of aptamer-2 on the GO/PtAu$_{\text{NPs}}$ was 57 nmol/mg according to simple calculations.

**Fig. S2.** Peroxidase-like activity of (a)GO, (b) naked GO@PtAu and (c) the aptamer-2 modified GO/PtAuNPs on the colorimetric reaction of TMB in the presence of H$_2$O$_2$. 
Fig. S3. Linear relationship between absorbance value of the oxidized TMB product at 652 nm and the concentration of GO/PtAuNPs. The error bars represent the standard deviation of three independent measurements.

Figure S3 provides the linear relationship between absorbance value of the oxidized TMB product at 652 nm and the concentration of GO/PtAuNPs. This research shows that with the increasing concentration of the superior catalyst, the catalytic rate of TMB is enhanced relatively.