Screening of inhibitors for oxidase mimics of Au@Pt NRs by catalytic oxidation of OPD

Electronic Supplementary Information

Experimental section

Chemicals and reagents. Sodium borohydride ($NaBH_4$), chlorauric acid ($HAuCl_4 \cdot 3H_2O$), cetyltrimethylammonium bromide (CTAB), potassium tetrachloroplatinate(II) (K_2PtCl_4), silver nitrate ($AgNO_3$), Poly-(sodium 4-styrenesulfonate) (PSS), poly(diallyldimethyl ammoniumchioride) (PDDAC) and L-ascorbic acid (AA) were all purchased from Alfa Aesar and used as received. 30% H_2O_2, o-phenylenediamine (OPD), 3,3',5,5'-tetramethylbenzidine (TMB), and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) were from Sigma-Aldrich (Milwaukee, WI). Other reagents and chemicals were at least analytical reagent grade and purchased from Beijing Chemical Reagent Company (Beijing, China). Milli-Q water (18 MΩ cm) was used for all solution preparations.

Synthesis of Au nanorods (NRs). Au NRs were synthesized by using a seed-mediated growth procedure.[7] CTAB-capped Au seeds were synthesized by chemical reduction of $HAuCl_4$ with $NaBH_4$: CTAB (7.5 mL, 0.1 M) was mixed with $HAuCl_4$ (100 μL, 24 mM), diluted with water to 9.4 mL, and stirred with a magnetic stirrer. Then, ice-cold $NaBH_4$ (0.6 mL, 0.01 M) was added. The solution color immediately turned from bright yellow to brown, indicating the formation of seeds. The Au seeds were used within 2 – 5 h. 120 μL seed solution was added to the growth solution consisted of CTAB (100 mL, 0.1 M), $HAuCl_4$ (2.04 mL, 24 mM), $AgNO_3$ (1.05 mL, 10 mM), and AA (552 μL, 0.1 M) to initiate the growth of Au NRs. After 12 hours, AA (55.2 μL, 0.1 M) was added under stirring twice with 40 min interval. The reaction mixture was reacted for 24 h. Au NRs were purified by centrifuging the solution at 12000 rpm for 5 min twice. The precipitate was collected and redispersed in deionized water, and the volume was reduced to 50 mL.

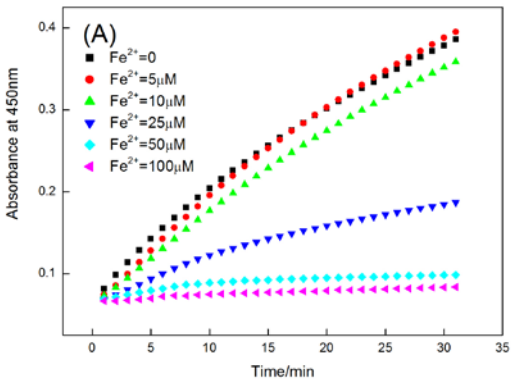
Synthesis of Au@Pt NRs.[8] Au NR solution (1 mL) was mixed with 75 μL of 2 mM $PtCl_4^{2-}$ aqueous solution. Then, 15 μL 0.1 M of AA was added and the total solution volume was diluted to 2 mL. The mixture was shaken vigorously and then placed in a 30°C water bath for 30 min. Within several minutes, the color of the solution changed from pink-red to dark gray, suggesting the formation of a Pt shell. Then 1 mL of 0.1 M CTAB was added.

Modification of the Au@Pt NRs with PSS. CTAB-coated nanorod solution (1mL, Au@Pt NRs) was centrifuged at 12000 rpm for 10 min, and the precipitate was dispersed in 0.5 mL PSS aqueous solution (2 mg mL^{-1} containing 6 mM NaCl). Then the solution was stirred magnetically for 3 h. After that, it was centrifuged at 12000 rpm for 10 min, and the precipitate was redispersed in water.

Characterization. The UV-vis-NIR absorption spectra were obtained from Varian Cary 50. Transmission electron microscopy (TEM) images were captured on a FEI TECNAI G2 F20 U-TWIN at an accelerating voltage of 200 kV.

Catalytic Oxidation of OPD in the absence and presence of inhibitors. 10 μL aliquot of OPD (0.1 M) with or without inhibitors was diluted by 3 mL of 0.1 M pH 4.5 PBS buffer. Then, 50 μL of PSS-coated NRs (5 nM) was added into the solution. UV-vis-NIR spectra were recorded after the addition of NRs at different times. The reaction temperature was kept at 37 °C.

Measurements of inhibition parameters. For irreversible inhibitors, k_{obs}/[I] is used to characterize inhibition degree. k_{obs} is the observed pseudo-first order rate of inactivation (determined as the negative slope of a plot of $\ln(v_t/v_0)$ versus time) and [I] is the concentration of inhibitor.



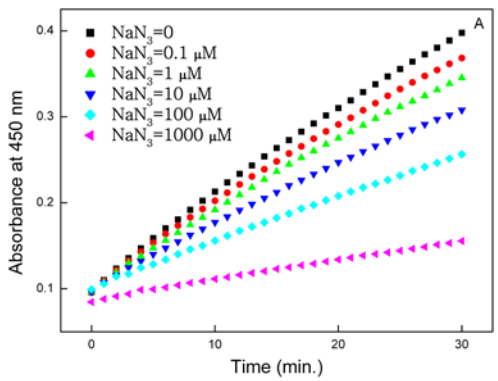


Fig. S1 Inhibition kinetics of of Au@Pt NRs by Fe^{2+}: (A) A_{450nm} vs. time, (B) $\ln(v_t/v_0)$ vs time, and (C) k_{obs} vs concentration of Fe^{2+}. Reaction conditions: 0.33 mM OPD, 0.083 nM Au@Pt NRs at 37 °C 0.1 M pH 4.5 PBS buffer.

For reversible inhibition, the inhibitor (I) binds to E and ES with the dissociation constants of Ki and Ki', respectively. In the presence inhibitor, each inhibitor concentration gives different values of apparent maximal velocity (V_{maxapp}) and apparent Michaelies-Menten constant (K_{appm}). In the plot of $1/V_{maxapp}$ vs inhibitor concentration, the intercept on the inhibitor concentration axis gives K_i'. In the plot of K_{appm}/V_{maxapp} vs inhibitor concentration, the intercept on the concentration axis gives K_i.

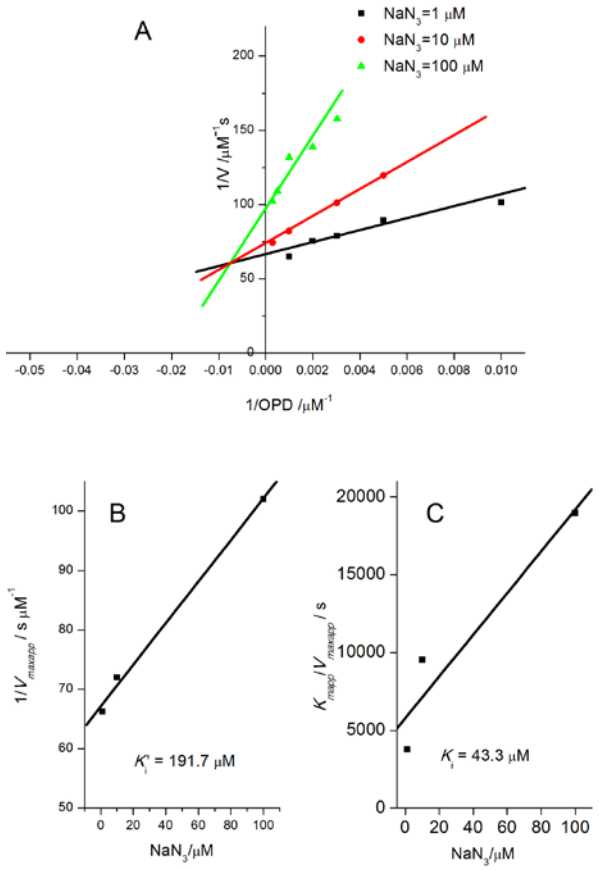


Fig. S2 Lineweaver-Burk plots in the presence of different concentrations of NaN_3 (A) and plots of $1/V_{maxapp}$ vs NaN_3 (B) and K_{appm}/V_{maxapp} vs NaN_3 (C). Reaction conditions: 0.017 nM Au @Pt NR s at different concentrations of OPD at 37 °C 0.1 M pH 4.5 PBS buffer.

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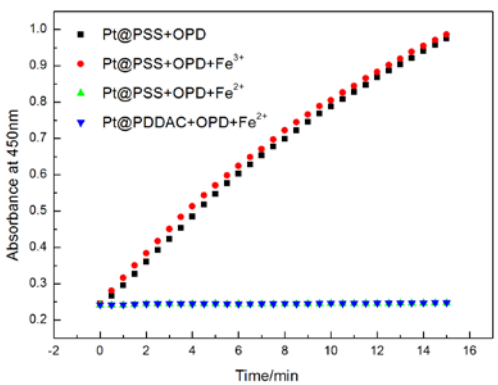


Fig.S3 Kinetics of OPD oxidation by Au@Pt N Rs without o r w ith inhibition. Reaction conditions: 0.33 mM OPD, 100 μM Fe^{2+} or Fe^{3+} with 0.083 nM Au@Pt NRs at 37 °C 0.1M pH 4.5 PBS buffer.

Screening of metal ions as inhibitors and detection of Hg^{2+} ions. 10 μL aliquot of OPD (0.1 M) and metal ions were diluted by 3 mL of 0.1 M PBS (pH = 4.5). Then, 50 μL of PSS-coated NRs (5 nM) were added into the solution. UV-vis-NIR spectra were recorded 30 min later after the addition of NRs. The reaction temperature was kept at 37 °C. For the detection of Hg^{2+} ions, reactions were terminated by adding stop solution (100 μL 10 M H_2SO_4).

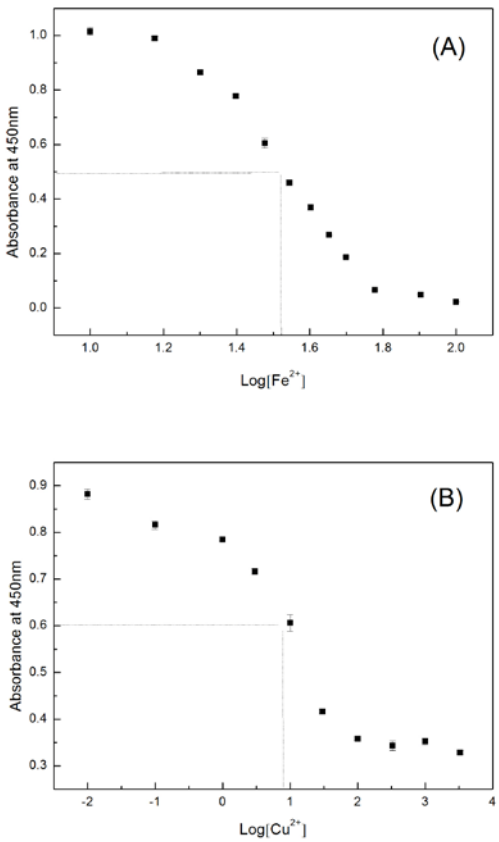
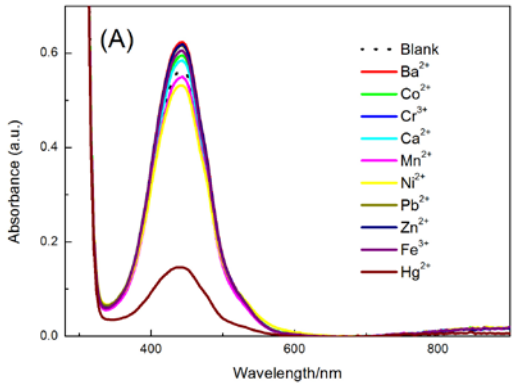


Fig. S4 Inhibitory effects of A) Fe^{2+} and B) Cu^{2+} on the activity of Au@Pt NRs.



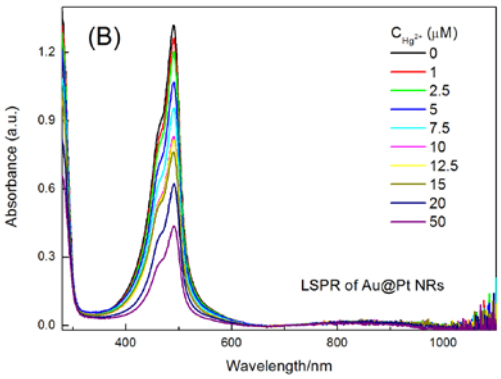


Fig. S5 Effects of (A) metal ions (100 μM) and (B) Hg^{2+} ions under different concentrations on the oxidase-like activity of Au@Pt NRs. Other reaction parameters: [OPD] = 0.33 mM, [Au@Pt NRs] = 0.083 nM at 37 °C in 0.1 M pH = 4.5 PBS buffer for 30 min.