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Electronic Supplementary Information

Colorimetric Assay of K-562 Cells Based Folic Acid-conjugated Porous Bimetallic Pd@Au Nanoparticles for Point-of-care Testing

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Materials and Apparatus

Chloroauric acid hydrated ($HAuCl_4 \cdot 4H_2O$, analytical grade), Sodium tetrachloropalladate (Na_2PdCl_4, ≥99.9%), Hexadecylpyridinium chloride monohydrate ($C_5H_5N(Cl)(CH_2)_{15}CH_3 \cdot H_2O$, HDPC, ≥99.0%), Ascorbic acid ($C_6H_8O_6$, AA, reagent grade), propargyl amine (95%), chloropropyl amine (98%), sodium azide, folic acid (≥97%), 1,1'-carbonyldiimidazole (≥97%), aminopropylazide and N, N-dimethylformamide (DMF, ≥99.9%) were all purchased from Aladdin Industrial Corporation. All the chemicals were used as received without further purification. All solutions were prepared using Milli-Q water (Millipore water purification system ≥18 MΩ, Milli-Q, Millipore, Billerica, MA) as a solvent. K-562 cell line was kindly provided from Qi lu Hospital of Shandong University. RPMI 1640 culture medium (500mL, GIBCO) was supplemented with fetal calf serum (10%, Sigma), penicillin(100 μg/mL), and streptomycin(100 μg/mL) at 37^{o}C in a humidified atmosphere containing 5% CO_2. The cells in exponential growth phase were collected and separated from the medium by centrifugation at 1000 rpm for 5min and then washed thrice with a sterile pH 7.4 PBS. The sediment was resuspended in 10mmol/L pH 7.4 PBS to obtain a homogeneous cell suspension. Cell number was determined on a Petroff–Hausser cell counter (U.S.A.).

Transmission electron microscopy (TEM) images were recorded on a JEOL 2010 transmission electron microscope operating at an accelerating voltage of 120 kV. The dynamic light scattering (DLS) measurements were conducted using a Brookhaven particle size and zeta potential analyzer at room temperature. Scanning electron microscope (SEM) images were obtained using a QUANTA FEG 250 thermal field emission SEM (FEI Co., USA). X-ray photoelectron spectra (XPS) were measured using a Thermofisher ESCALB 250 X-ray photoelectron spectrometer. The phase characterization was performed by X-ray diffraction(XRD) using a D8 advance diffractometer system equipped with Cu Kα radiation (BrukerCo., Germany).

UV-vis absorption spectra were recorded on a Shimadzu UV-3101 UV/vis spectrophotometer (Shimadzu, Japan). Fluorescence measurements were performed

on a LS-55 spectrofluorimeter with 1.0 cm quartz cell (Perkine Elmer, USA). The 96 well micro-plates were read in a TriStar LB 941 Multimode Reader (Berthold Technologies GnbH & Co. KG, Bad Wildbad, Germany).

Synthesis of porous Pd@Au Nanostructures

The Pd@Au Nanostructures with perpendicular pore channels was prepared by wet-chemical synthesis according to the reported method with corresponding modification[1]. To prepare the porous Pd@Au nanostructures, a mixture solution containing 2.0 mL Na_2PdCl_4 solution (10 mmol/L), 0.5 mL $HAuCl_4$, solution (10 mmol/L), 0.1 g HDPC, and 25 mL H_2O were added into a vial. After homogeneous mixing, a freshly prepared 1.5 mL ascorbic acid solution (0.1 mmol/L) was added quickly into the mixture solution under gentle shaking. After the vial was sealed, the resulting mixture was standed for 3 hours at 35 ºC. The obtained black porous Pd@Au nanostructures could be easily isolated from the solution by centrifugalization and was purified by washing with copious amount of Milli-Q water. Finally, it was washed with absolute ethanol and dried in a vacuum.

Synthesis of propargyl-Pd@Au nanoparticles

The preparation procedure of alkynyl-Pd@Au was fabricated based on the conjugation of Pd@Au nanopartcles with propargyl amine via the noncovalent bond between palladium nanoparticles and available amine groups of propargyl amine[2](Fig. S1 a). The Pd@Au nanoparticles (0.01 mg of Pd@Au) were added into an eppendorf tube containing propargyl amine (3.6 mg, 10 mmol) in 0.25 mL of DMF. The mixture was incubated at room temperature for 4 h. The final reaction mixture was purified by dialysis using 8-10 K molecular weight cut off dialysis bag, against water and phosphate buffered saline (PBS) solution.

Synthesis of azide-functionalized folic acid

Firstly, 3.8 g of chloropropyl amine (40.0 mmol) and 7.8 g sodium azide (120.0 mmol) were transferred into a 100 mL round bottom flask containing 50 mL of water and

heated at 75 ^{o}C for 18 h[3]. The reaction mixture was concentrated by rotary evaporation at the condition of high vacuum, and 2.5 g of KOH was gradually added into the mixture, and the mixture was extracted by using diethyl ether. Subsequently, the result product was dried and concentrated with anhydrous sodium sulphate. Finally, the pure aminopropylazide was obtained through flash column chromatography using 4% ethyl acetate in petrolium ether as an eluant. 1,1'-carbonyldiimidazole (CDI) (9.0 mg, 0.054 mmol) was added into an eppendorf tube containing folic acid (20.0 mg, 0.045 mmol) in dry DMF (1 mL) and incubated for 2 h at 35 ^{o}C. Aminopropylazide (5.0 mg, 0.049 mmol) were added into the mixture and incubated the mixture solution at room temperature for 30 h. The product was then cetrifuged and washed to remove excess starting materials. Finally, the azide-functionalized folic acid(Fig. S1 b) was dissolved in 1 mL of DMF.

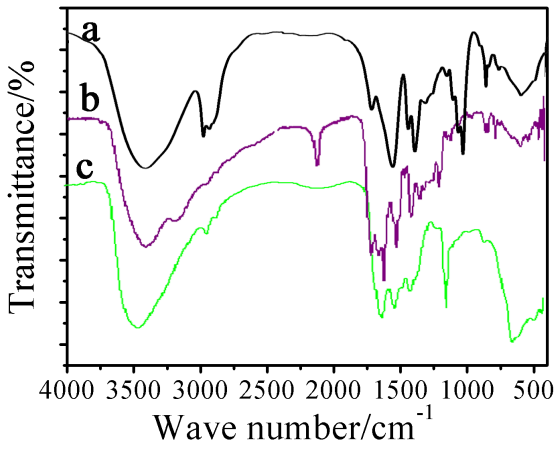

Fig. S1 Fourier Transform infrared spectroscopy, (a): propargyl-Pd@Au nanoparticles; (b) azide-functionalized folic acid; (c): folic acid functionalized Pd@Au nanoparticles

Synthesis of folic acid functionalized Pd@Au nanoparticles by Click chemistry

The propargyl-Pd@Au nanoparticles (0.030 g) were added into azide-functionalized folic acid (0.004 g) in 0.5 mL of DMSO and the solution was vortexed for 1 min. A 100 μL freshly prepared solution of 0.1mol/L $CuSO_4$ and 0.2mol/L ascorbic acid was added and the mixture was vortexed for 2min.This mixture was stored at room temperature for 6 h[4].The final reaction mixture was purified by dialysis using 8-10 K molecular weight cut off dialysis bag, against deionized water and phosphate buffered

saline (PBS) solution. The purified folic acid functionalized Pd@Au nanoparticles (Fig. S1 c) were stored in refrigerator for further characterization and application.

Cultures of K562 cells

Various amount of Cells were plated in 96 well plate and incubated overnight at room temperature. Each well was washed with washing buffer two times. A 100 µL of folate-Pd@Au NPs (0.05 mg/mL) was added into this well and allowed to incubate at room temperature for 1 h. Subsequently, each well were rinsed three times with PBS buffer. To each well, 100 µL of 0.1 mmol/L TMB and 100 µL of 0.1 mmol/L H_2O_2 was added, and incubated in PBS (pH 6.4) for 5 minutes to allow development of the blue color. The absorbance of each well was determined immediately using a microplate reader set to 652 nm. In another set of experiment, similar immunoassay were carried out using increasing number of MCF-7 cells keeping the concentration of folate-Pd@Au NPs constant.

Mechanism of Peroxidase-Like of Pd@Au NPs.

The procedure of experiment was implemented by using 50 mmol/L H_2O_2, 0.625 mmol/L terephthalic acid, and different concentrations of the Pd@Au NPs incubating in 0.2 mmol/L PBS buffer (pH 6.4) at room temperature for 6 h.

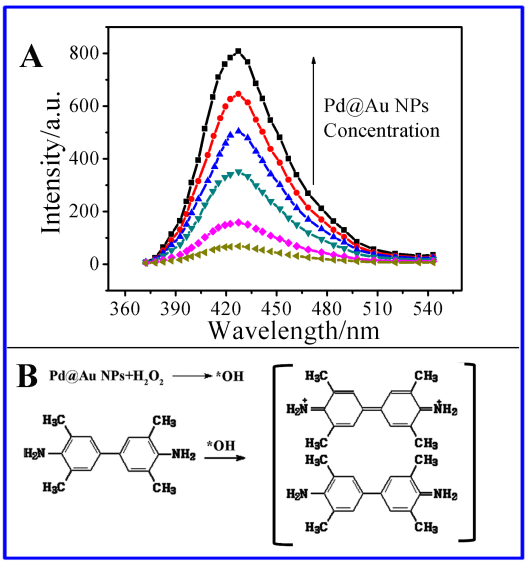

Fig. S2 (A) The effect of the Pd@Au NPs on the formation of hydroxyl radical with terephthalic acid as a fluorescence probe, (B)Mechanism of TMB color change

Optimization of Experimental Conditions

The catalytic activity of Pd@Au NPs is similar to HRP, and both is also dependent on surrounding conditions (including pH, temperature, and H_2O_2 concentration). The oxidation of TMB was much faster in nearly neutral solutions than in strong acid or basic solutions with the presence of H_2O_2 and Pd@Au NPs, i.e. pH=6.4. Thus, the pH 6.4 buffer solution was selected as the optimal acidity to get a high catalytic activity(Fig. S3). The concentration of H_2O_2 was another important factor influencing the sensitivity of the assay. The concentration of H_2O_2 was at the range of 0.05~ 0.1 mmol/L, the change in the absorbance was not obvious. Within the working range, the absorbance kept stable and high absorbance. To acquire a wide linear range and low detection limit of the developed method, 0.1 mmol/L H_2O_2 was employed for the development of visible color in the following assay(Fig. S4). TMB could be readily oxidized by H_2O_2 in the prescence of catalyst Pd@Au NPs in the range of 20 to 60 ^oC with Pd@Au NPs catalyst. The absorbace of the TMB/H_2O_2 solution kept stable. Considering the practical application of the proposed system in clinical assays, all experiments were carried out at room temperature (about 25 ^oC). Thus, room temperature was taken as the optimal reaction temperature(Fig. S5). In view of the stability and catalytic activity of the Pd@Au NPs, 6.4 was set as the optimal pH value. The optimal temperature and H_2O_2 concentration were room temperature and 0.1 mmol/L, respectively.

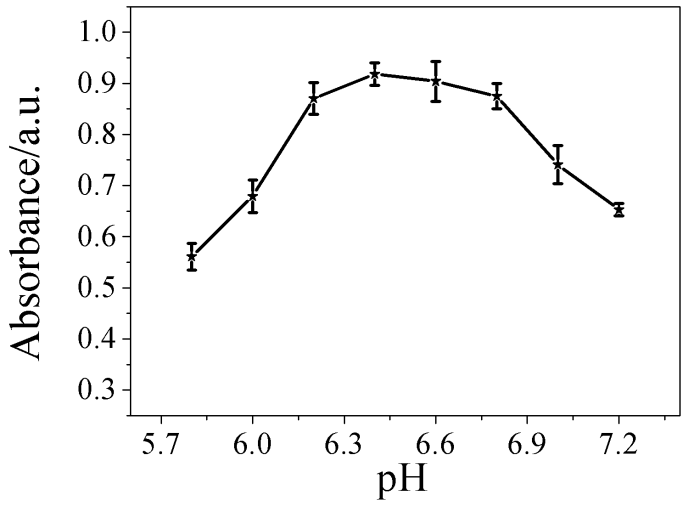

Fig. S3 Effect of pH, 10000 Cells/mL of K 562

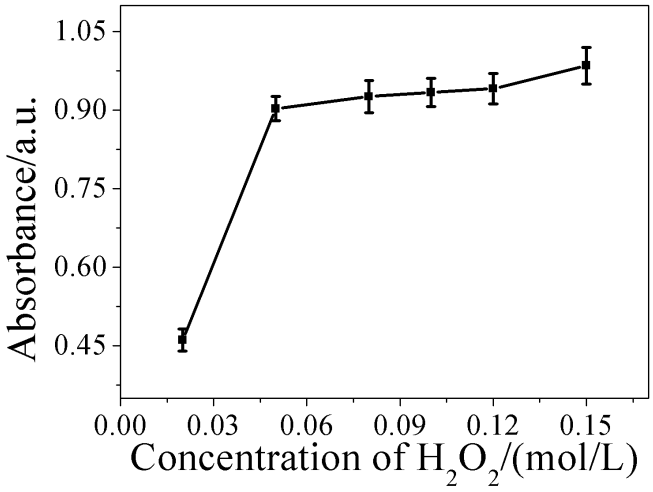

Fig. S4 Effect of temperature, 10000 Cells/mL of K 562

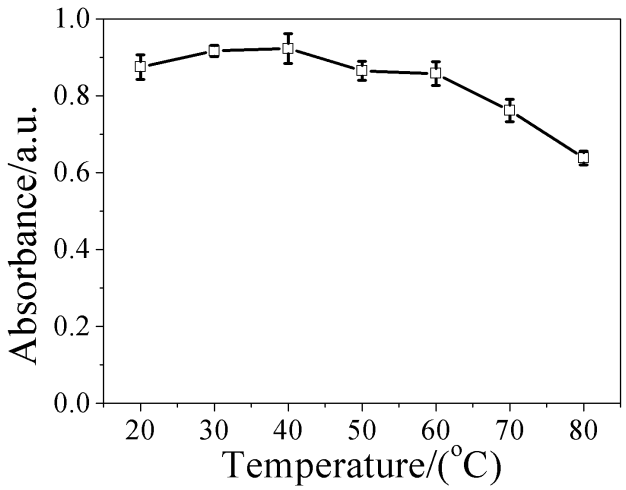

Fig. S5 Effect of H_2O_2 concentration, 10000 Cells/mL of K 562

Robustness of Pd@Au NPs.

The robustness of the Pd@Au NPs in wide pH and temperature ranges is key to extend their potential applications. Pd@Au NPs, one of inorganic materials, were expected to be more robust than natural enzymes. Under the condition of pH lower than 4.0 or temperatures greater than 40 °C for 2 h, HRP activity sharply declines. By contrast, Pd@Au NPs were robust when they were incubated at a rigorous condition of pHs (2-11) and wide temperatures (0-90 ^{o}C) for 2 h. The robustness of Pd@Au NPs made them suitable for a broad range of applications in the biomedicine, clinical chemistry and environmental chemistry fields.

Detection of K-562 Cells in Human Blood

Table S1 Detection of K-562 cells in human blood

Sample	Proposed method (cells/mL)	RSD (%)	Add target cells (cells/mL)	Detected cells (cells/mL)	Rescovery (%)	Reference method (cells/mL)
1	650	5.7	2000	2130	106.5	670
2	2810	3.8	5000	4940	98.8	2910
3	26730	4.1	10000	10430	104.3	26040

Reference

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