Supplementary Information

Pt Nanozyme for O_2 Self-Sufficient, Tumor Specific Oxidative Damage and Drug Resistance Reversal

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Experimental Section

Materials: Tetraethyl orthosilicate (TEOS, 99%), (3-mercaptopropyl) trimethoxysilane (MPTS, 95%), 3-aminopropyl trimethoxysilane (APTMS, 97%), H_2O_2, and all other reagents were of analytical reagent grade and used as received. calcein-AM, PI，Hoechst 33342 and 5-Carboxyfluorescein (FAM) were provided from Sigma-Aldrich (USA), and Qiancheng Technology Co. Dulbecco's modified Eagle's medium (DMEM), Roswell Park Memorial Institute (1640), trypsin, fetal bovine serum (FBS) and MTT, penicillin-streptomycin were purchased from GIBCO Invitrogen Corp. Ultrapure water (18.2 MΩ; Millpore Co., USA) was used in all experiments and to prepare materials.

Synthesis of Au@SiO_2 and PtAu@SiO_2: SiO_2 nanospheres were synthesized using a seed growth method (Stöber).[1,2] Then 40 mL of $HAuCl_4$ (0.25 mM) and sodium citrate (0.25 mM) were taken into an ice-water bath, and then fresh $NaBH_4$ solution was added under constant stirring. After the solution was changed from bright yellow to burgundy, it was left for 15 minutes to get Au NPs. To get Au@SiO_2 Nanoparticles, 2 mL of the above-prepared aminated SiO_2 nanospheres solution was added to a 50 mL glass vial, and 15 mL Au NPs solution was added under stirring condition. 30 minutes later, the solution was centrifuged and washed three times to remove excess Au NPs and the purified Au@SiO_2 nanoparticles were dispersed in water. The above-prepared Au@SiO_2 were added to a flask

and stirred at room temperature. Subsequently, H_2PtCl_6 (0.2 mL, 19.3 mM) and sodium citrate (1 mL, 1%) were added and stirred for 15 minutes so that H_2PtCl_6 was fully adsorbed on the surface of the Au@SiO_2. Finally, fresh NaBH4 solution was added and the reaction was stirred for a further 15 minutes. The solution was centrifuged and washed for three times to obtain PtAu@SiO_2.

Characterization of PtAu@SiO_2: Morphology of PtAu@SiO_2 was observed via TEM (JEM-2100 microscope). The hydrodynamic size and zeta potential of PtAu@SiO_2 in deionized water were measured by a Nano-ZSZEN3600 (Malvern Instruments) at 25°C. Energy-dispersive X-ray EDS mapping images (Si, Au and Pt) were analyzed by Four-Detector designed Super X Energy Dispersive Spectrometry. X-ray photoelectron spectroscopy (XPS) spectra were analyzed by Thermo Fisher Scientific ESCALAB 250Xi Spectrometer Electron Spectroscopy (America).

pH Responsive Release of Platinum Ion of PtAu@SiO_2: 0.5 mL PtAu@SiO_2 solution was positioned in a dialysis bag (MWCO 500 Da) and dialyzed in 10 mL of PBS buffer with different pHs of 6.5 and 7.4. The dialysate was collected at preset times and fresh PBS buffer was added. The leached platinum ion of different dialysate was determined via ICP-MS.

Oxidase Mimetic PtAu@SiO_2: The TMB oxidation by PtAu@SiO_2 was conducted in the absence of H_2O_2. 50 μL TMB were respectively added into the same amount of PtAu@SiO_2 (20 mg/L, 100 μL) and Au@SiO_2 solution (PBS buffer, pH 6.5 or 7.4). After 5 minutes, the photos were obtained. In order to verify the color change sensitivity of TMB in different pHs, PtAu@SiO_2 was incubated with TMB in PBS buffer with different pHs of 4, 5, 6, 7 and 8. Three PtAu@SiO_2 solution samples were placed in pH 6.5 PBS buffer, one was purged with nitrogen, one was purged with oxygen and the other placed in air. After 20 minutes, samples (100 μL) were added to each tubes containing 100 μL TMB. The color change was recorded.

PtAu@SiO_2 Mediated H_2O_2 Decomposition and Oxygen Production: Various concentrations of PtAu@SiO_2 (0.2, 1, 2 mg/L) were incubated with H_2O_2 (10 mM), respectively. The oxygen evolution over time of the above samples was recorded through a portable dissolved oxygen meter (Shanghai Ray magnetic JPB-607A). Besides, the oxygen generation of PtAu@SiO_2 incubated with different concentrations of H_2O_2 (0, 1, 10, 50 mM) for 10 min was also observed.

PtAu@SiO_2 Mediated ROS Generation: The generation of ROS was measured via fluorescence spectroscopy using DCFH-DA as the sensor. 8 mL of 0.01 M NaOH was mixed with 2 mL of DCFH-DA in methanol (0.5 mg/mL) in the dark for 30 min at room temperature to chemically hydrolyze

DCFH-DA to DCFH. Then, 40 mL of the PBS buffer (10 mM, pH 7.4) was added to stop the reaction. The stock solution of DCFH was kept in a -20°C in the dark before use. For fluorescence spectrum test, collected PtAu@SiO_2 and equal amount Au@SiO_2 was redispersed in PBS buffer with different pHs (6.5, 7.4 and 6.5+Vc, 10 mM, 10 mg/L), then 100 μL of NaOH pretreated DCFH-DA was added. Then the fluorescence spectroscopy of the mixtures at a preset time was recorded via fluorescence spectrophotometer (Ex: 485 nm; Em: 495~600 nm). To distinguish the type of ROS, the electron spin resonance (ESR) technique was employed to monitor ROS signals from PtAu@SiO_2 using BMPO and DMPO as the spin-trapping agents for $\bullet O_2^-$ and •OH, respectively. Spectra of spin trapped $\bullet O_2^-$ and •OH were acquired by mixing 5 μL BMPO (500 mM) or DMPO (5 μL, 500 mM) with 100 μL PtAu@SiO_2 or Au@SiO_2 in different pHs of 6.5 and 7.4.

Intracellular ROS Production: HeLa cells were seeded into culture dishes in 5% CO_2 at 37°C for 24 h. Then, Fresh H_2DCF-DA (10 mM) was added into each well and the cells were incubated in 5% CO_2 at 37°C for 20 min. After the cells were washed with PBS for three times, the cells were treated with PtAu@SiO_2, Au@SiO_2 in different pH medium (pH 6.5, 7.4) and PtAu@SiO_2 + Vc (4 μL, 1 mg/mL) in pH 6.5 medium. Subsequently, the cells were washed with PBS and then were immediately captured by the confocal laser scanning microscopy (CLSM).

Cytotoxicity Assays: HeLa Cells and A549/DDP were seeded in 96-well plates in 5% CO_2 at 37°C for 24 h, respectively. Then the cells were treated with PtAu@SiO_2, Au@SiO_2 in different pHs of 6.5 and 7.4 and PtAu@SiO_2 in the presence of Vc (8μL, 1 mg/mL) in pH 6.5, respectively. After 40 h incubation, the medium was replaced with fresh medium containing 30 μL MTT (5 mg/mL) each well. The supernatant was replaced with 150 μL DMSO to dissolve the formazane at post of 4 h incubation. The optical density (OD) values at 490 nm were recorded by a microplate reader (Bio-Rad, Model 550, USA). The relative cell viability was calculated by the following formula: cell viability (%) = $OD_{(sample)}/OD_{(control)} \times 100\%$. $OD_{(sample)}$ was the OD value in the presence of sample and $OD_{(control)}$ was the optical density in the absence of sample.

Calcein AM/PI Assay: HeLa Cells and A549/DDP were seeded 35 mm diameter dishes in 5% CO_2 at 37°C for 24 h, respectively. Then the cells were treated with PtAu@SiO_2, Au@SiO_2 in different pHs of 6.5 and 7.4 and PtAu@SiO_2 in the presence of Vc (8 μL, 1 mg/mL) in pH 6.5, respectively. Cells were incubated for 40 h. The cells were detected observed using CLSM after co-staining with calcein AM (2 μM) and PI (3 μg/mL) for 20 min.

Cytotoxicity in Different O_2 Concentrations: For studying the remission of hypoxic environment of PtAu@SiO_2, the A549/DDP cells were seeded in 96-well plates and 35 mm diameter dishes in normoxic (21% O_2, 5% CO_2, 74% N_2) or hypoxic (5% O_2, 5% CO_2, 90% N_2) condition for 24 h. After that, PtAu@SiO_2 and cisplatin, at the indicated concentrations, were added to the cell culture medium and incubated with cells for 40 h. Finally, the ability to alleviate hypoxia was judged by MTT assay and observed via CLSM after co-staining with calcein AM (2 μM) and PI (3 μg/mL) for 20 min.

Investigating the uptake mechanism: In order to track the entry of PtAu@SiO_2 into cells in real time, fluorescent dyes (5-Carboxyfluorescein, FAM) were attached to PtAu@SiO_2 (named PtAu@SiO_2-FAM) through a reaction between the amino group on the surface of the SiO_2 and the carboxyl group of the fluorescein dyes. A549 cells were pre-incubated in DMEM for 1 h at 37 °C with different pathway inhibitors such as chlorpromazine (10 mg/L, clathrin-mediated endocytosis inhibitor), genistein (50 mg/L, caveolae-mediated endocytosis inhibitor), and amiloride (4 mg/L, macropinocytosis inhibitor). Moreover, A549 cells were pre-cultured at 4 °C for 1 h to investigate the influence of energy on the cell internalization process. After that, the cells were washed with PBS, followed by incubation with PtAu@SiO_2-FAM for 4 hours at 37 ℃. The mean fluorescence intensity of cells treated with various aforementioned inhibitors was compared with the control group (treated without any cellular uptake inhibitors) by flow cytometry. To obtain the ratios of cellular uptake, the cells treated as above than were incubated with fresh aqua regia overnight and then diluted with water for ICP-MS analysis. For cell colocalization, after different inhibitors and subsequent PtAu@SiO_2 treatment, the cells were washed with PBS in order to remove PtAu@SiO_2 that was not absorbed. Subsequently, the cells were co-cultured with 1640 containing H33342 dye（10 mg/L） for 30 minutes. Finally，the cells were washed with PBS and then were immediately captured by the confocal laser scanning microscopy (CLSM), nucleus (blue, Ex:405 nm, Em:440~480 nm); PtAu@SiO_2-FAM (green, Ex:488 nm, Em:495~550 nm).

Cell Cycle progression by Flow Cytometry: A549/DDP cells (in the exponential growth phase) were seeded in 6-well plates in normoxic (21% O_2, 5% CO_2, 74% N_2) or hypoxic (5% O_2, 5% CO_2, 90% N_2) condition for 24 h. After that, the cell culture medium was replaced by fresh culture medium (pH 6.5) with or without PtAu@SiO_2. Then, the cells were repositioned in a hypoxic environment for 24 hours. Similarly, the cells under normoxia were treated with PtAu@SiO_2-containing medium (pH 6.5)

and cultured under normoxia for 24 hours. After PtAu@SiO_2 treatment in different environments, the cells were washed, collected, re-suspended in ice-cold PBS and fixed using 70% ethanol at 4°C for over 24h. Thereafter, cells were centrifuged, re-suspended in ice cold PBS and then stained with PI using the cell cycle and apoptosis analysis kit (Beyotime Biotechnology, China) at 37°C in the dark for 30 minutes. Cells were then detected using the BD FACSCalibur CellSorting System and results were analyzed using FlowJo 7.6.1 software.

Gene Analysis: A549/DDP cells (in the exponential growth phase) were seeded in 6-well plates in hypoxic (5% O_2, 5% CO_2, 90% N_2) condition for 24 h. After that, the cell culture medium was replaced by fresh culture medium (pH 6.5) with or without PtAu@SiO_2. Then, the cells were repositioned in a hypoxic environment for 24 hours. After that, the cells were washed three times and send to Shanghai Majorbio Bio-pharm Technology Co.,Ltd for eukaryotic tran-scriptome sequencing.

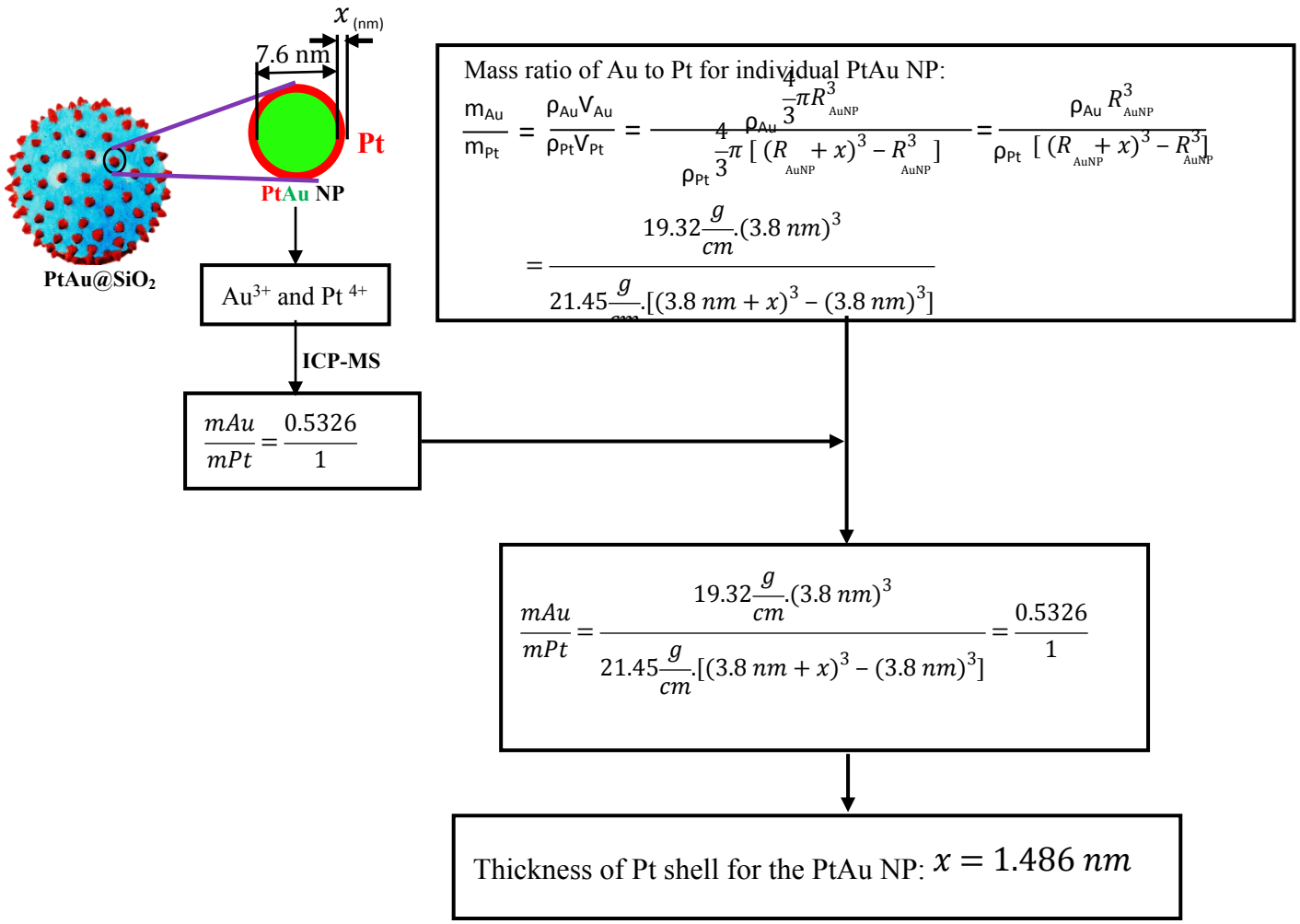

Figure S1. Schematic showing the estimation of Pt shell thickness (x nm) for the PtAu NPs on the $PtAu@SiO_2$ shown in Figure 1.

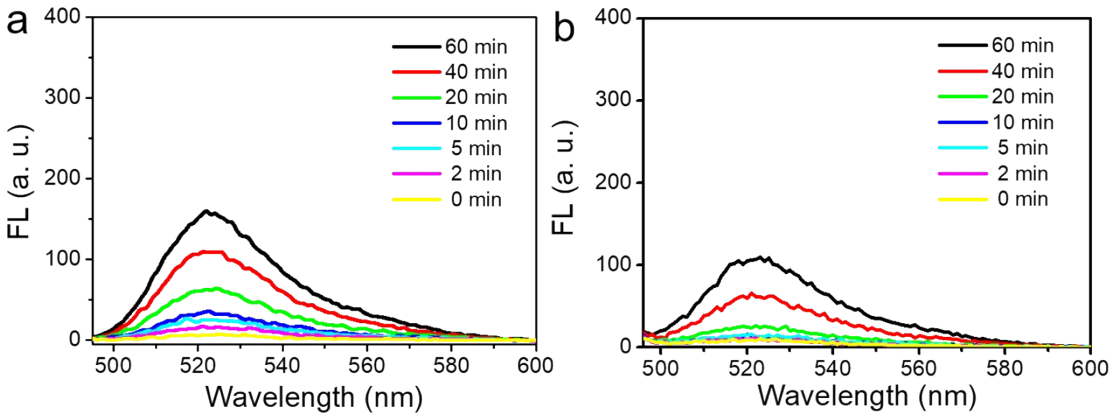

Figure S2. Fluorescence spectra of DCF by adding a) Au@SiO_2 over time at pH 6.5 and b) PtAu@SiO_2 over time at pH 6.5 in the presence of Vc.

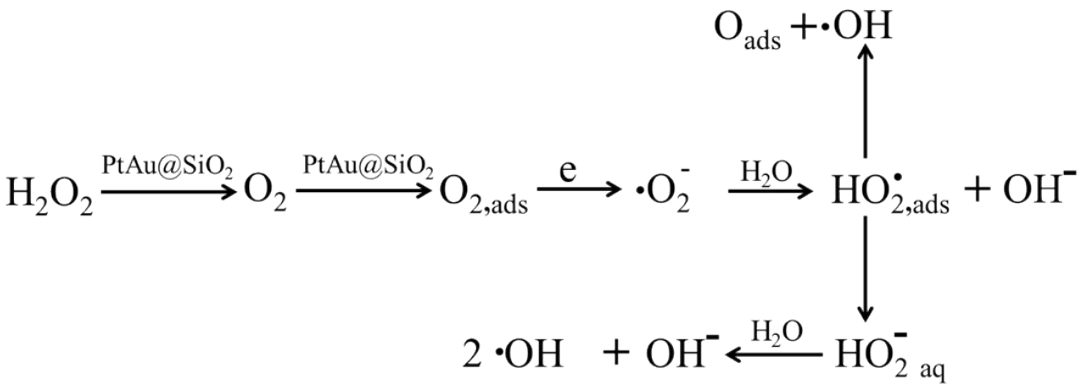

Figure S3. The potential process of PtAu@SiO_2 catalyzed generation of superoxide anion and hydroxyl radical in a hypoxic tumor microenvironment. PtAu@SiO_2 nanozyme firstly catalyze H_2O_2 into O_2 and then catalyze O_2 to generate superoxide anion and hydroxyl radical under hypoxic conditions.

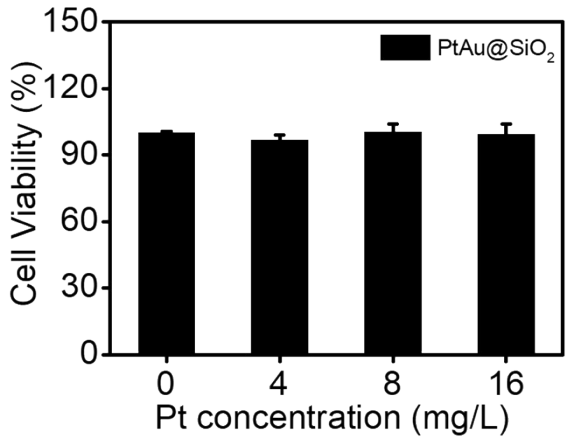

Figure S4. Cell viability of PtAu@SiO_2 against COS7 cells at pH 7.4.

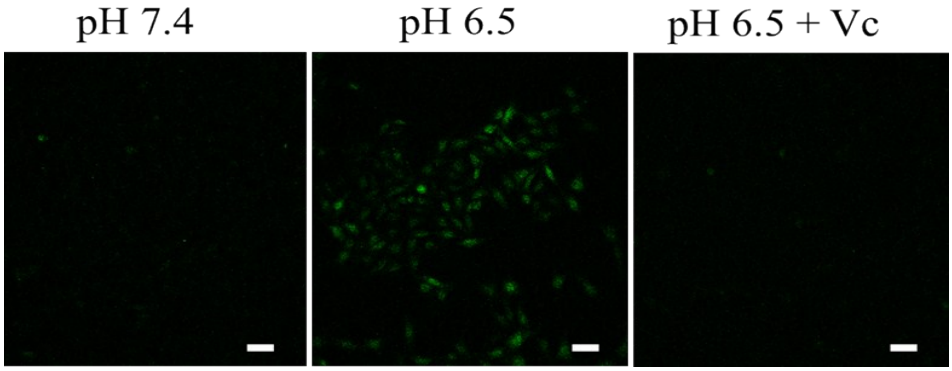

Figure S5. . The production of ROS in A549/DDP cells treated with PtAu@SiO_2 under different environments (pH 7.4, pH 6.5 and pH 6.5+Vc). DCF fluorescence (green) depicted intracellular ROS. The scale bare was 50 μm.

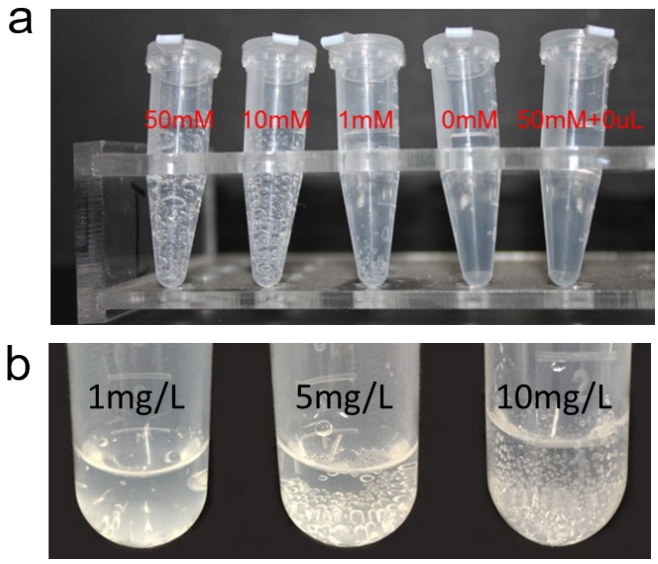

Figure S6. Digital photos of PtAu@SiO_2 producing oxygen at pH 6.5 a) after reaction with increasing concentrations of H_2O_2: 0 mM, 1 mM, 10 mM, 50 mM; PtAu@SiO_2: 3 mg/L; and b) after reaction with increasing concentrations of PtAu@SiO_2: 1 mg/L, 5 mg/L, 10mg/L, H_2O_2: 10 mM.

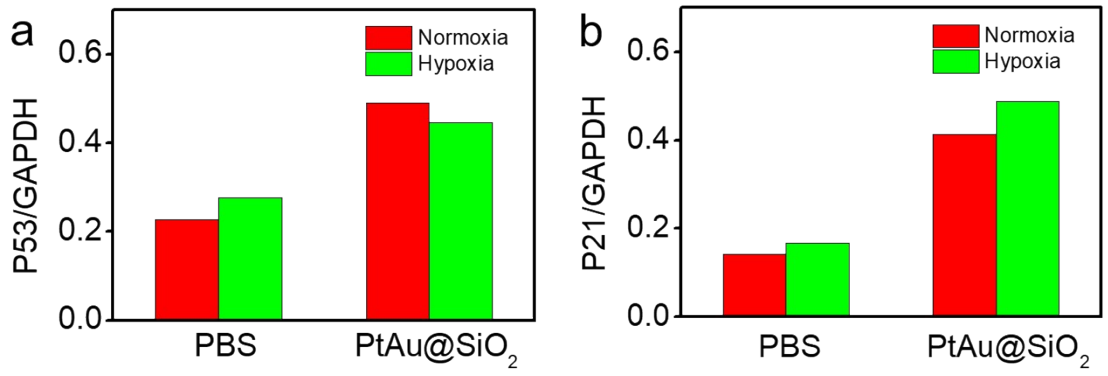

Figure S7. Quantitative analysis of light intensities of a) P53 and b) P21 protein expression, as the ratio of protein to GAPDH from Western blot results.

References

1. L. Huang, Q. Wu, J. Wang, M. Foda, J. W. Liu, K. Cai, H. Y. Han, *Chem. Commun.* **2014**, *50*, 2896.
2. L. Wu, X. P. Li, K. Shao, S. Y. Ye, C. Liu, C. J. Zhang, H. Y. Han, *Anal. Chim. Acta* **2015**, *887*, 192.