Electronic Supplementary Information

One-step rapid synthesis of fluorescent platinum nanoclusters for cellular imaging and photothermal treatment

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Experimental section

Chemicals and instruments

Chloroplatinic acid ($H_2PtCl_6 \cdot 6H_2O$), glutathione and ascorbic acid were purchased from sinopharm chemical reagent company (analytical grade). Trypsin solution, DMEM medium, fetal bovine serum and antibiotics were purchased from Sigma Aldrich. Stock solutions were freshly prepared in sterilized phosphate buffer (PBS) (0.1 M, pH=7.2) and stored in the dark at 4 °C. All the other regents used in this report were analytical pure.

The UV-vis and fluorescence (FL) spectra are recorded on a Biomate 3S spectrometer (Thermo Co. Ltd., US) and a RF-5301PC fluoremeter (Shimadzu, Tokyo, Japan), respectively. The transmission electron microscopic (TEM) and high–resolution TEM images are obtained on a JEM-2100 TEM (JEOL, Japan) at an applied accelerating voltage of 200 KV. The X-ray photoelectron spectroscopic data are recorded on a PHI 5000 VersaProbe X-ray photoelectron spectrometer (XPS) with an Al Kα = 280.00 eV excitation source. The mass spectroscopy (MS) is recorded on a LCQ Fleet electrospray ionization (ESI) mass spectrometer(Thermo, USA).

Preparation and characterization of Pt NCs

Pt NCs were synthesized by utilizing 0.1 mol of the oxidant, chloroplatinic acid ($H_2PtCl_6 \cdot 6H_2O$) and different concentration of the stabilizer glutathione (GSH) (i.e., 0.1, 0.1, 0.05 mol) and reducing agent ascorbic acid (i.e., 0.05, 0.1, 0.1mol), with solution volume ratio of 1:1:1, where the reaction was conducted under 37 °C. The Pt NCs stabilized by GSH were precipitated using ethanol (1:3). Later, the mixture was

centrifuged at 8000 rpm for 10 min and the supernatant was discarded. The Pt NCs were prepared by vacuum drying under 37 °C for 3h to remove ethanol. Finally, the sample were dispersed in PBS (pH=7.2) and also in MEM medium for further use and stored at 4°C .

The morphology of the nanocrystals was characterized by JEM-2100 High Resolution Transmission Electron Microscopy (HRTEM). FTIR spectra of GSH ,VC and Pt NCs were taken by using a thermo fisher scientific FTIR spectrophotometer (American, Nicolet Co.) in the interval 4000-400 cm^{-1}. Meanwhile, absorption and emission spectra of the nanocrystals were measured at room temperature (ca. 20 °C) by using a Hitachi-4100 UV-Vis absorption spectrophotometer and a Hitachi-7000 fluorescent spectrometer respectively. The average size of Pt NCs was about 1.4 nm, and the apparent lattice spacing about 0.39 nm.

Cell cultures

Cancer cell lines like HepG2, HeLa and nomal cell lines like L02 were maintained in glass flasks in DMEM medium containing 10% fetal bovine serum and antibiotics (100 mg/ml streptomycin and 100 IU/ml penicillin) and cultured at 37 °C in 5% CO_2.

In vitro cytotoxicity studies of Pt NCs

The viability of cells was measured by the MTT method. HeLa cells in the log phase were seeded in 96-well plates at a concentration of 1.0×10^4 cells/well and incubated overnight at 37 °C in a 5% CO_2 humidified environment. Then the cells were treated with different concentration of Pt NCs (dissolved with DMEM medium). Control groups were cultivated under the same condition without Pt NCs. All samples were cultured for 48 h, then 20 mL of 5 mg/mL MTT was added and incubated for additional 4 h. Subsequently, the plates was centrifuged at 1000 rpm for 10 min and the supernatant was discarded, followed by addition of 150 mL of DMSO into each well and gentle shaking in the shaker incubator at 37 °C for 10 min. After this, the optical density (OD) was recorded at 492 nm. Cell viability was expressed as follows: cell viability (%) = $[A]_{test}/[A]_{control} \times 100\%$, where [A] represents the absorbance.

Confocal microscopy studies.

Cancer cell lines like HepG2 ,HeLa and normal cell lines like L02 (10^5 cells in 1 mL) in DMEM medium containing 10% fetal bovine serum and antibiotics (100 mg/ml streptomycin and 100 IU/ml penicillin) were seeded in 6 wells plate with coverslips . After 24 hours, the medium was removed and the wells were washed three times with PBS. Pt NCs decorated with GSH dissolved in PBS were added to each well, which were incubated for 24 hours at 37°C in a 5% CO_2. The medium was then removed and the cells were washed three times with PBS. Then samples were incubated with 500 µL of 4% paraformaldehyde in PBS for 15 minutes. The supernatant was removed and the samples covered with coverslips were maintained in the dark at 4°C. Confocal microscopy was performed on a Leica TCS SP5 Leica Microsystems GmbH microscope (Wetzlar, Germany) using the Leica Application Suite advanced fluorescence 2.0.2 software for data acquisition. Images were acquired using a 63x oil immersion lens with argon laser set at 405 nm to detect Cells with Pt NCs.

Inverted fluorescence microscopic studies

The Pt NCs were added into HeLa cells' suspensions in the presence of UV (400nm) irradiation /or infrared irradiation (IR-635nm) for 15 minutes. In the meantime, the cell suspensions with no Pt NCs treatment but infrared (IR) irradiation and cell suspensions with Pt NCs without irradiation were taken as controls. All samples were maintained for 24 h at 37◦ C in a 5% CO_2 humidified environment. The fluorescence was captured by an IX71 inverted fluorescence microscope with the excitation wavelength at 488 nm.

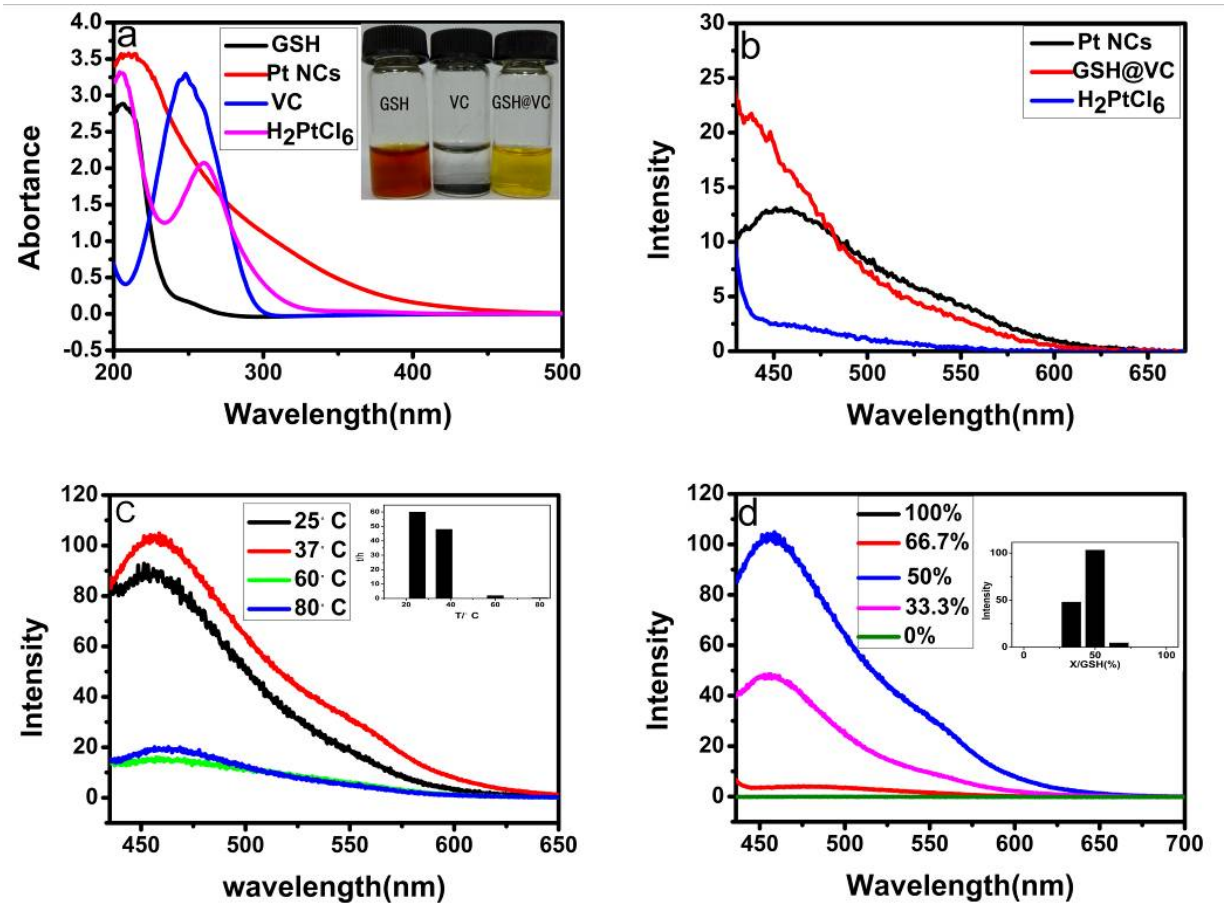

Figure S1. (a) UV-Vis absorption spectra of Pt NCs (red solid line), H_2PtCl_6 (pink solid line), GSH (black solid line), VC (blue solid line); the product colour of GSH or VC with H_2PtCl_6 under 80 ℃ (inset of a); (b) Emission spectra of the blue-emitting Pt NCs (excitation wavelength λ = 365 nm); (c) The fluorescence intensity and time needed to formation (inset) of Pt NCs under different temperature; (d) The fluorescence spectra of Pt NCs in the presence of different relative concentration of GSH among the total of GSH and VC under 37 ℃ with related histogram (inset) .

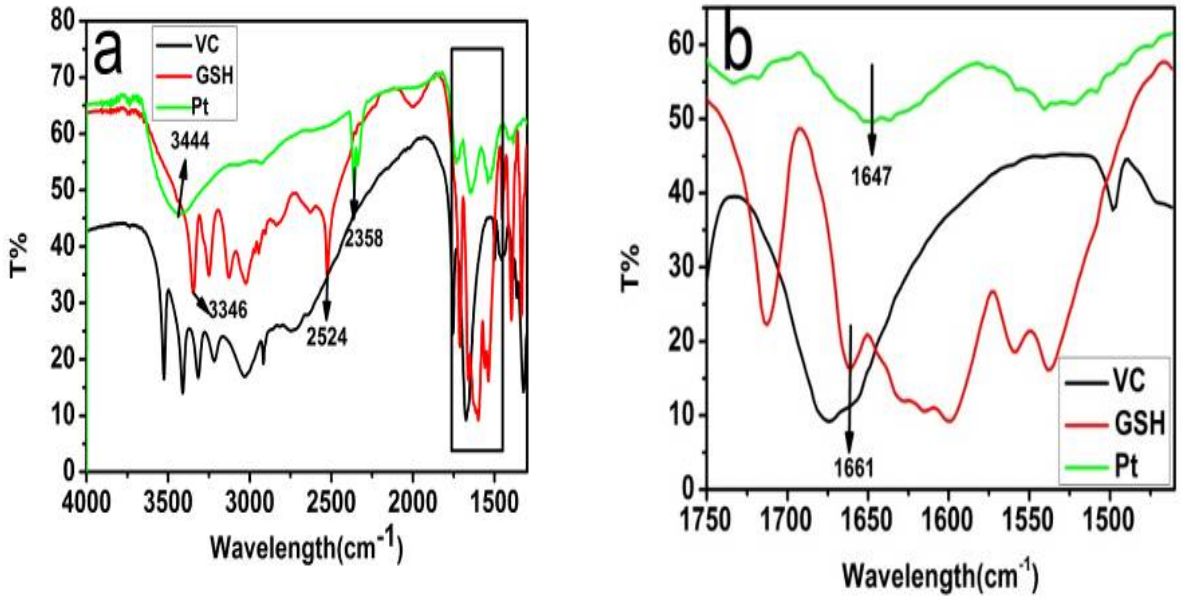

Figure S2. (a) FTIR spectra of Pt NCs (green solid line), GSH (red solid line), VC (black solid line) ; (b). Zoom of (a) over the range 1450-1750 cm^{-1}.

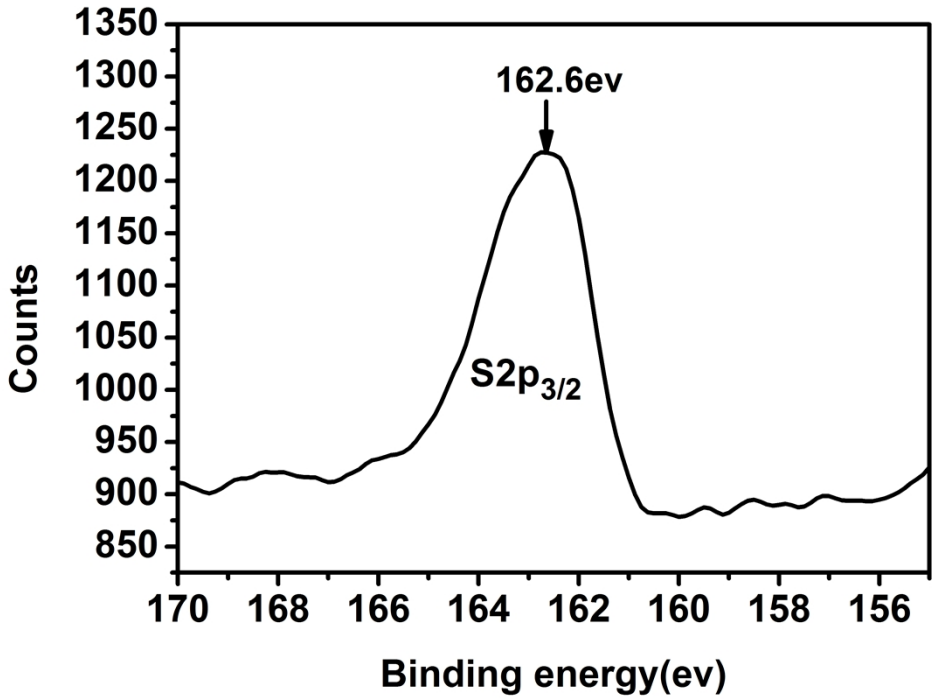

Figure S3. XPS spectra of Pt NCs. S $2p_{3/2}$ established the presence of platinum bound to sulfur with a peak at 162.6 eV.

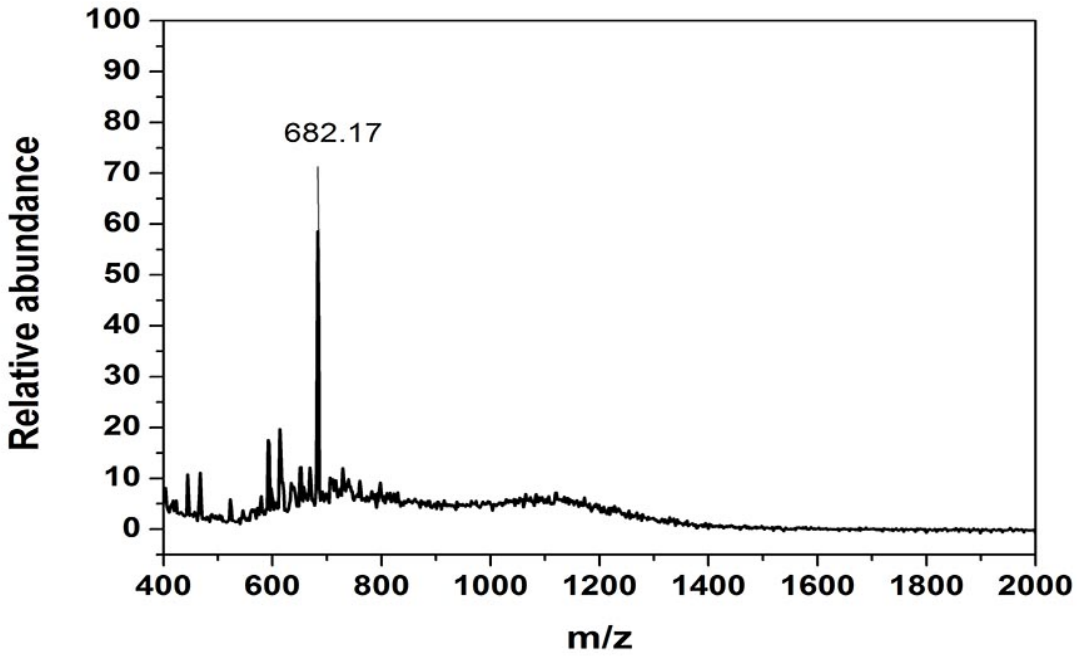

Figure S4. The ESI-MS curve for Pt nanoclusters. The mass spectrum shows that Pt NCs mainly consist of three platinum atoms per NC, i.e., with the high abundant components of $[Pt_3(GS)_7]^{4-}$ at m/z=682.17. The high abundance for $[Pt_3(GS)_7]^{4-}$ among all peaks indicates that Pt and GSH are prone to form complexes at a ratio of 3:7.

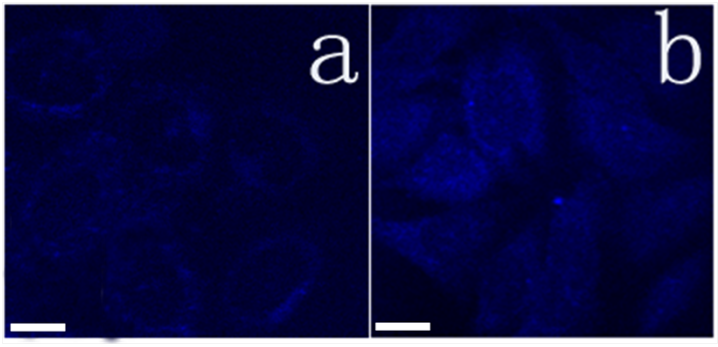

Figure S5.Typical confocal laser scanning microscope images of the intracellular accumulation of Pt NCs in HeLa cells. HeLa cells were incubated (a) in the absence of Pt NCs and (b) in the presence of Pt NCs (150 μg/mL) for 24h at 37◦ C. (Scale bar are 25 μm).

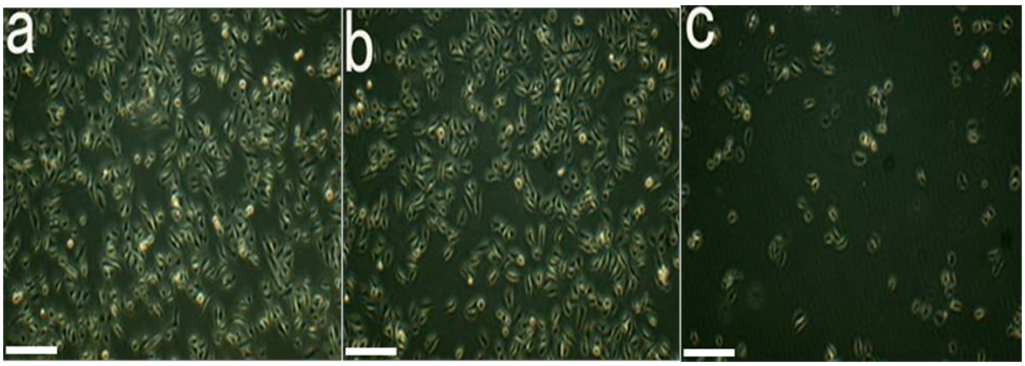

Figure S6. Light microscope images of HeLa cells treated with / or without Pt NCs / IR irradiation, showing apparent morphological changes of the HeLa cells treated with SM-7032B LED laser (ca. 635 nm) for 10 mins. (a) (control) Typical image of HeLa cells before laser treatment of IR irradiation. (b) Typical image of HeLa cells treated with Pt NCs (150μg/10^6 cells) alone, (c) Typical image of HeLa cells treated with Pt NCs (150μg/10^6cells) after laser treatment of IR irradiation for 10 mins. (Scale bar are 10 μm).