Electronic Supporting Information

for

Gold Nanorods for Platinum based prodrug delivery

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Materials and Methods

The complexes *cis*-[$Pt(NH_3)_2Cl_2$] and *c,c,t*-[$Pt(NH_3)_2Cl_2(O_2CCH_2CH_2CO_2H)_2$] (**1**) were synthesized by literature method.[1,2] Chloroauric acid ($HAuCl_4 \cdot 3H_2O$), sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3$), ascorbic acid (AA), 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide Hydrochloride (EDC·HCl) and N-hydroxysuccinimide (NHS) were obtained from Aldrich. Hexadecyltrimethylammonium bromide (CTAB) was obtained from Sigma. Succinic anhydride (SA) was purchased from Alfa aesar and used without purification. Rhodamine B isothiocyanate (RBITC) and 4',6-diamidino-2-phenylindole (DAPI) were purchased from Cytoskeleton Inc. (Denver, USA). Ultrapure water (18.2 MΩ) from Millipore Milli-Q Biocel purification system containing a 0.22 μm filter was used for all experiments. Phosphate buffered saline (PBS) containing 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4 was adjusted to pH 7.4. Methyl-PEG_{5000}-TA was as a gift provided by Dr. Yucai Wang. All other chemicals were of reagent grade and used without further purification.

UV-Vis plasmon spectra of GNRs were measured using an Aglient 8453 UV-Visible spectrophotometer. Transmission electron microscopy (TEM) of the gold nanorods was performed on a JEOL 2010 transmission electron microscope with an accelerating voltage of 200 kV. Pt content was measured on an X Series 2 Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Thermo fisher Scientific). Bruker AV300 NMR spectrometer (300 MHz) was used for ^{1}HNMR analysis.

Synthesis of PEG-GNRs: The GNRs were prepared via the method invented by Murphy et al.[3] Gold nanoparticle seeds were prepared by adding 0.6 mL of ice-cold solution of 10 mM sodium borohydride to 10 mL of 2.5×10^{-4} M gold chloride solution prepared in 0.1 M CTAB solution under vigorous stirring. The yellow color changed immediately to brown indicates the formation of gold nanoparticle seeds. The solution was further stirred for 10 min. This seed solution was used for the synthesis of gold nanorods after aging for at least 2 hours at 37°C in water bath. For preparation of GNRs, solutions were added to a 200 mL conical flask in following order: 95 mL 0.1 M CTAB solution, 1 mL 10 mM silver nitrate solution, 5 mL 10 mM chloroauric acid and then 0.5 mL of 0.1 M ascorbic acid. This solution was homogenized by gently shaking before adding a 0.12 mL of seed solution. The mixture was

homogenized and then left undisturbed overnight (14-16 hours) at 37°C in water bath. The violet-brown colored gold nanorods solution was collected by centrifugation. The pellet was resuspended and centrifugation twice at 14000 rpm for four minutes in order to remove excess CTAB and other salts. The GNPs was diluted to an optical density (O. D.) of 1.0–1.2.

Amine-terminated oligoethyleneglycol chains were tethered onto nanorods by in situ dithiocarbamate formation according to the literature.[4] An aqueous suspension of CTAB-coated nanorods (3 mL, O.D. 1) was treated, while stirring, with a 1 mL 10 mM O, O'-bis (2-aminoethyl) octadecaethylene glycol. The solution was adjusted to pH 9.5 by NaOH, followed by adding 0.1 mL saturated CS_2 solution (28 mM). The mixture was stirred for 12 hours. The amine-coated GNRs were collected by centrifugation in 14000 rpm for four minutes. The pellet was resuspended gently in 250 μM 5-kDa methyl-PEG-TA and the solution was stirred for overnight at room temperature for methyl-PEG-TA binding. The solution was centrifuged in 14000 rpm for four minutes. The pellet was resuspended in water, and the resulting conjugate solution was ready for Pt (IV) loading.

Synthesis of Pt-PEG-GNRs: The Pt-PEG-GNRs was synthesized by standard amide coupling reactions. In a typical reaction, a 1.0 mM aqueous solution of NHS (20 μL) was added to an equal volume of an aqueous 1.0 mM solution of EDC, and the resulting solution was allowed to stand at room temperature for 10 min. To this solution, 0.8 molar equivalent of compound **1** (40 μL) was added and stirred for 10 minutes before adding PEG-GNRs solution. The mixture was stirred for 24 hours at room temperature. The resulting solution was dialyzed exhaustively against ultrapure water (7000 kDa molecular weight cutoff). This procedure yielded stable and well-dispersed Pt-PEG-GNRs with absorption at λ_{max} 708 nm. The Pt content in Pt-PEG-GNRs was subsequently determined by platinum ICP-MS. The TEM samples were prepared by pipetting a drop of the solution onto a 230 mesh copper grid coated with carbon and allowing the sample to dry in air or in an oven at 44°C before measurements.

Cytotoxicity studies

Cell Line and Cell culture: The cancer cell line HeLa, A549 and MCF-7 (ATCC) were maintained in RPMI 1640 media (Gibco, USA) supplemented with 10% fetal calf serum

(FBS, HyClone, USA) at 37°C with 5% CO_2 in a 95% humidified atmosphere. For all experiments, cells were seeded to provide 90% confluence in 6-, 24-, or 96-well plates and grown for 24 hours.

Cell Fixing Solution: Paraformaldehyde (4.0 g) and NaOH (0.4 g) were dissolved in 100 mL of ultrapure water. 1.68 g NaH_2PO_4 was added to the solution and the pH was adjusted to 7.5 - 8.0 by NaOH and H_3PO_4. Sucrose (4.0 g) was added and the resulting solution was stored at 4°C until use.

MTT assay: All the solutions for the MTT assay were freshly prepared in sterile PBS before use. Platinum concentration was quantitated by atomic absorption spectroscopy. Cells were seeded on a 96-well plate in 100 μL media and incubated for 24 hours before adding drugs. The cells were then treated with Pt-PEG-GNRs or cisplatin at varying concentrations. After incubation for 72 hours at 37°C, the media were removed, and cells were washed with PBS and then incubated with cell culture medium containing 20% 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). After 2 hours incubation at 37°C in 5% CO_2, 100 μL lysis buffer was added to each well. (lysis buffer was prepared: 20 g Sodium dodecyl sulfate (SDS) dissolved in 50 mL ddH_2O and supplemented with 50 mL N, N-Dimethylformamide, pH 4.7). Cells were further incubated overnight and the absorbance was measured at 570 nm using a Bio-Rad 680 microplate plate reader. Cell viabilities at various Pt concentrations were presented as the percentage of control sample without drug treatment. Each experiment was repeated three times and the average values were taken in the analyses.

Fluorescence imaging from Confocal laser scanning microscopy (CLSM): HeLa cells at a density of 5×10^4 cells per well were grown on the glass coverslips in 24-well plates and maintained at 37°C in 5% CO_2 for 24 hours prior to treating with Pt-PEG-GNRs conjugates, which were labeled with RBITC on the surface. After 2 hours incubation, the medium was removed. The cells were washed thrice with RPMI 1640 medium and twice with Millipore water, fixed with cell fixing solution for 10 minutes at room temperature and then washed

with Millipore water again. Nuclei were stained by DAPI according to the standard protocol provided by the supplier. After staining, the cells were washed thrice with PBS and mounted on microscope slides for imaging by Leica TCS-SP5 spectral confocal laser scanning microscope (CLSM, Leica Microsystems, Germany, with two-photon system). Excitation wavelengths were 364 and 543 nm for DAPI and RBITC respectively.

Cellular uptake measured by flow cytometry HeLa cells were seeded in 24-well plates at a density of 15×10^4 cells per well in 0.5 mL RPMI 1640 medium and incubated in a humidified 5% CO_2 atmosphere for 24 hours. The original medium was replaced by RBITC-labeled Pt-PEG-GNRs containing RPMI 1640 medium. The cells were incubated for 2 hours at 37°C, and then washed thrice with RPMI 1640 medium and twice with PBS, and harvested by trypsin treatment. The harvested cells were suspended in PBS and centrifuged at 1000 rpm for 5 minutes. The supernatants were discarded and the cell pellets were washed with PBS to reduce the background fluorescence from the medium. After two cycles of washing and centrifugation, cells were resuspended with 200 μL cell fixing solution. RBITC-positive cells were enumerated by FACS Calibur flow cytometer (BD Biosciences, USA), and the results were analyzed with WinMDI 2.9 software.

References

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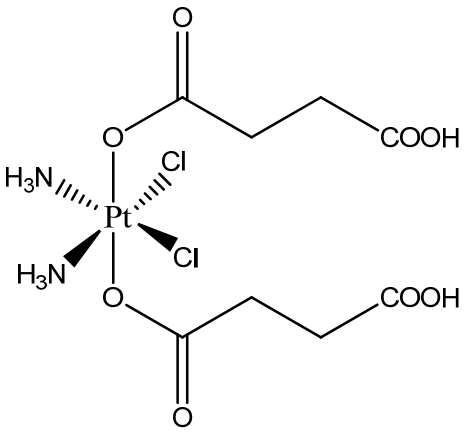
[3] Murphy, C. J.; Sau, T. K.; Gole, A. M.; Orendorff, C. J.; Gao, J.; Gou, L.; Hunyadi, S. E.; Li, T. J. Phys. Chem. B. 2005, 109, 13857.

[4] Ling Tong, Yan Zhao, Terry B. Huff, Matthew N. Hansen, Alexander Wei, and Ji-Xin Cheng. Adv. Mater. 2007, 19, 3136-3141

(A)



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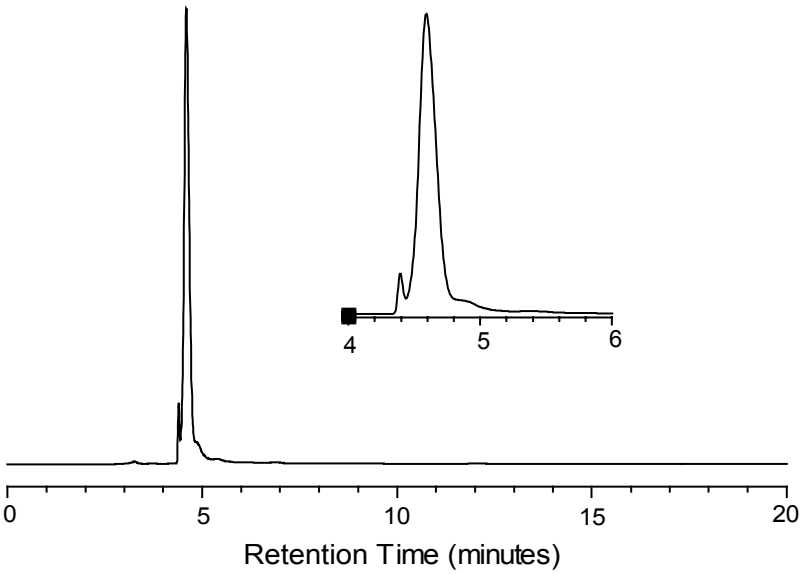

Figure S1. (A) The structure of prodrug *c,c,t*-[$Pt(NH_3)_2Cl_2(O_2CCH_2CH_2CO_2H)_2$]. (B) HPLC trace of *c,c,t*-[$Pt(NH_3)_2Cl_2(O_2CCH_2CH_2CO_2H)_2$] eluted by 90% H_2O and 10% acetonitrile.

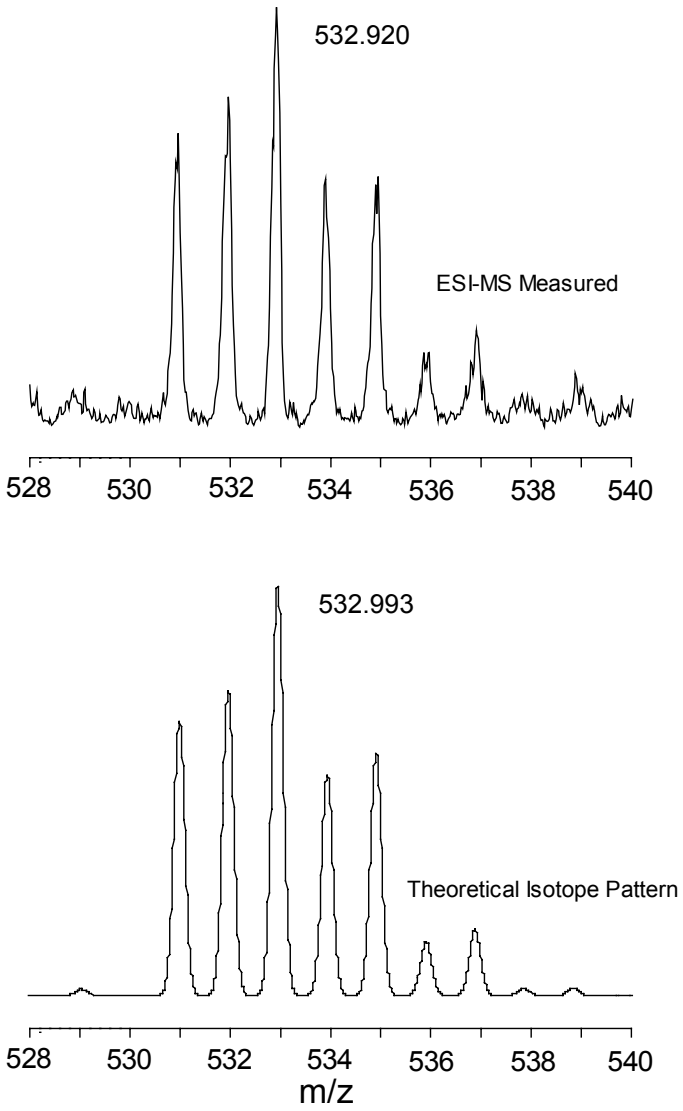

Figure S2. ESI-MS of *c,c,t*-[$Pt(NH_3)_2Cl_2(O_2CCH_2CH_2CO_2H)_2$] in the negative mode. Conformation: *c,c,t*-[$Pt(NH_3)_2Cl_2(O_2CCH_2CH_2CO_2H)_2$-H]$^-$, formula: $[C_8H_{16}N_2O_8Cl_2Pt_1]^-$. Up-portion: measured ESI-MS spectrum, m/z = 532.920; Low-portion: theoretically calculated isotope pattern, m/z = 532.993.

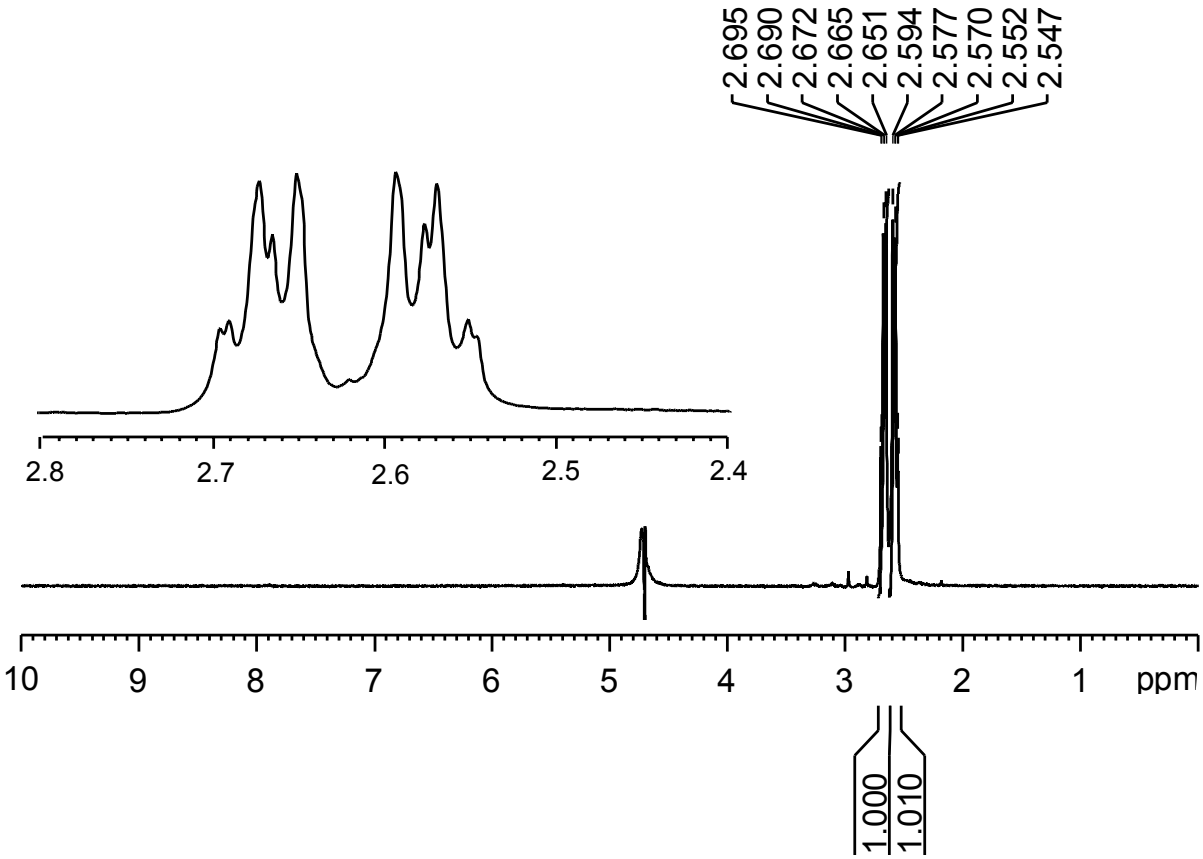

Figure S3. ^{1}H NMR of *c,c,t*-[$Pt(NH_3)_2Cl_2(O_2CCH_2CH_2CO_2H)_2$] in D_2O. An expended view is inserted in the figure.

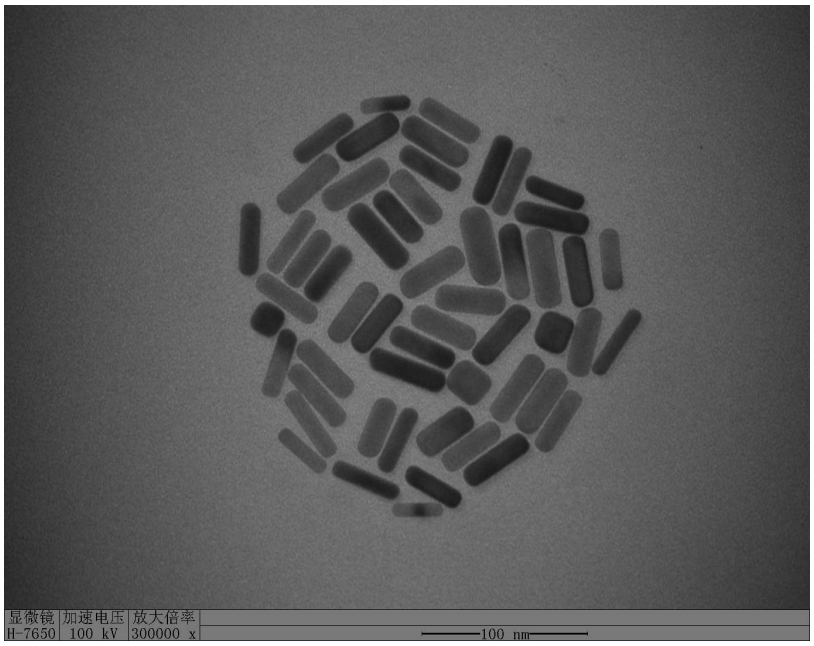

Figure S4. TEM of gold nanorods (45 nm×13 nm). Scale bar in figure equals 100 nm.

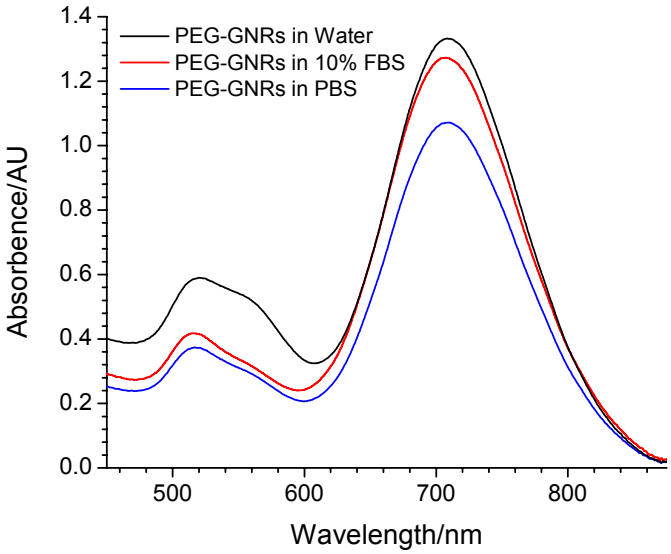

Figure S5. The UV-Vis spectra analysis of the stability of PEG-GNRs. Samples were incubated at 37 °C for 72 hours. Black line: PEG-GNRs in pure water; Red line: PEG-GNRs in 10% FBS; Blue line: PEG-GNRs in PBS.

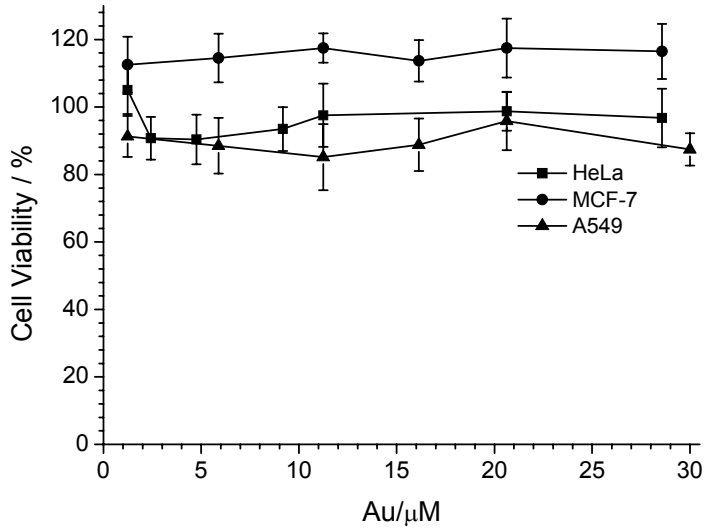

Figure S6. Cytotoxicity of drug vector (PEG-GNRs) on cancer cells. (■) HeLa cells, (●) MCF-7 cells, and (▲) A549 cells.

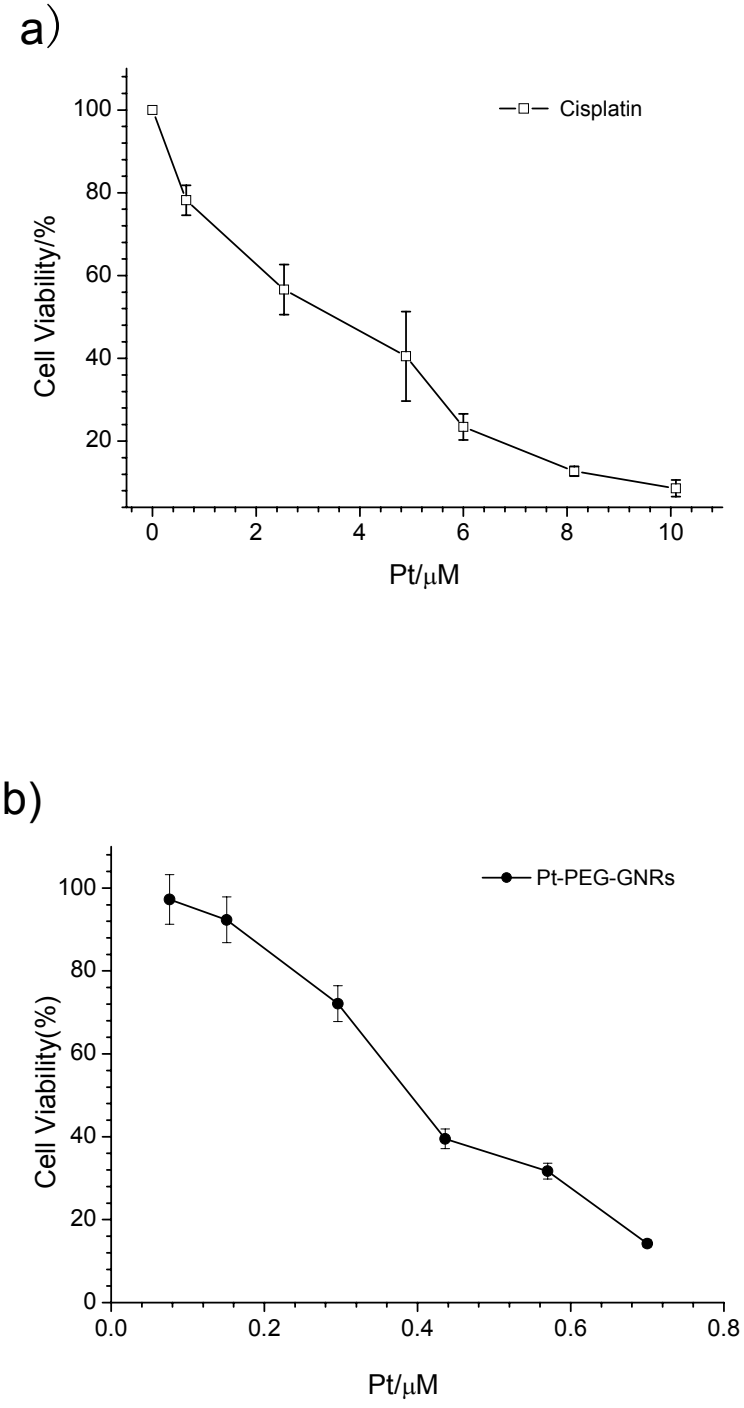

Figure S7. Cytotoxicity of Pt-PEG-GNRs and cisplatin on HeLa cells. (a) cisplatin; (b) Pt-PEG-GNRs.

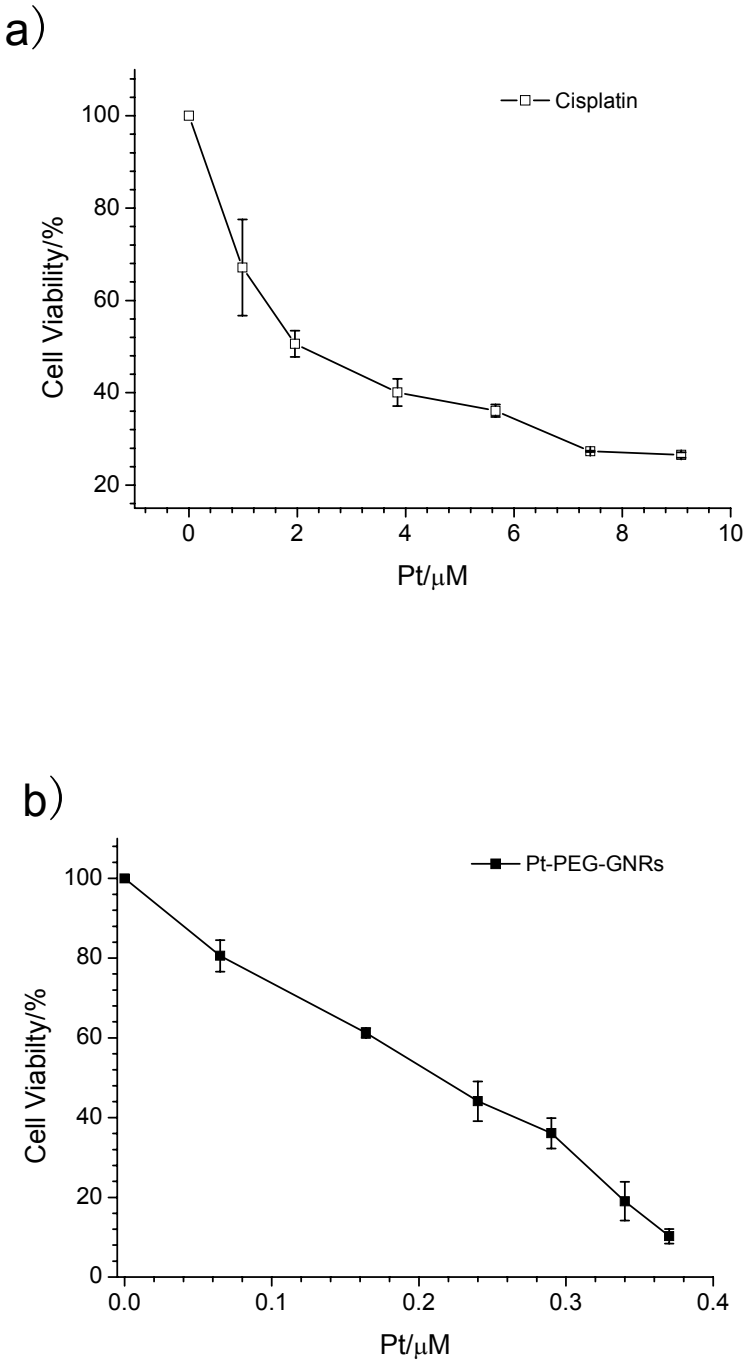

Figure S8. Cytotoxicity of Pt-PEG-GNRs and cisplatin on A549 cells. (a) cisplatin; (b) Pt-PEG-GNRs.

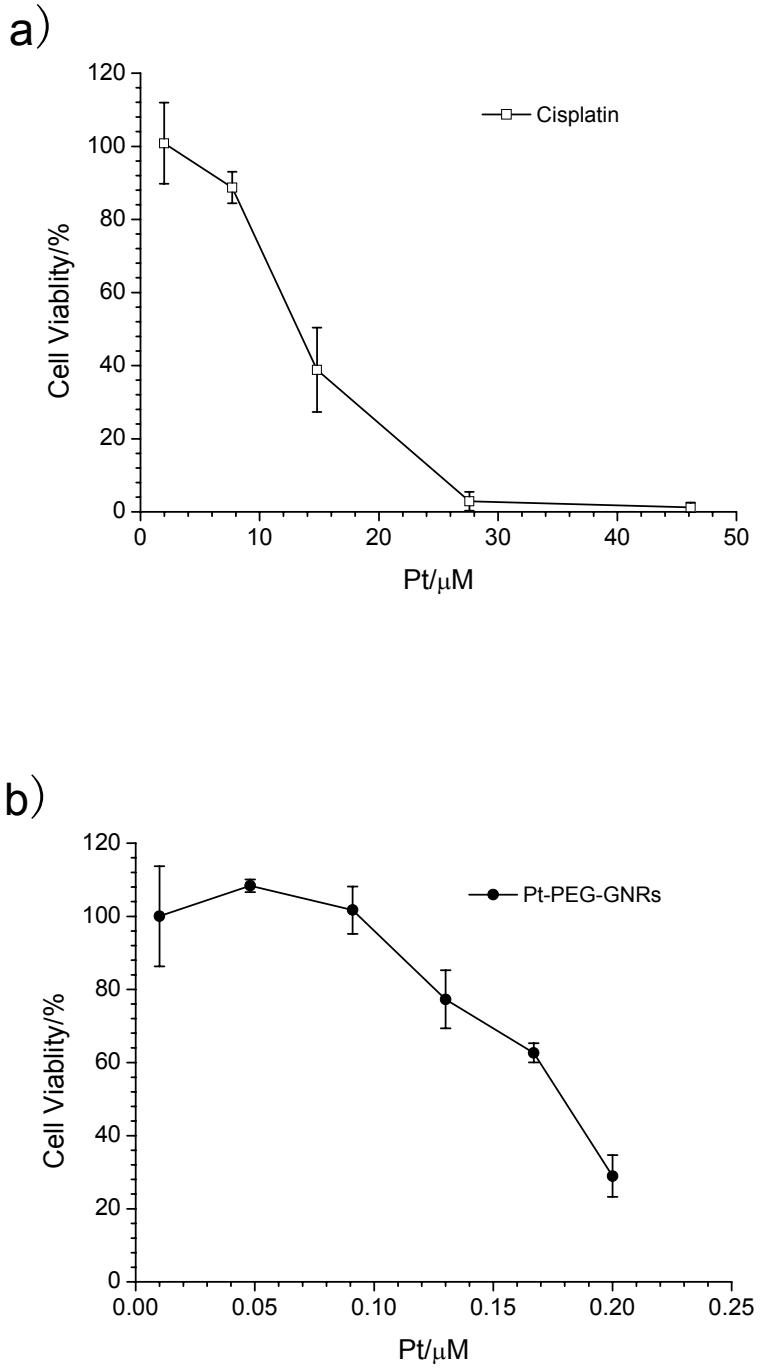

Figure S9. Cytotoxicity of Pt-PEG-GNRs and cisplatin on MCF-7 cells. (a) cisplatin; (b) Pt-PEG-GNRs.