Supporting Information for

A Dual Targeting Cyclodextrin/Gold Nanoparticle Conjugate as a Scaffold for Solubilization and Delivery of Paclitaxel

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Experimental Section

Materials. *β*-CD was recrystallized from water twice and dried in vacuo at 90 °C for 24 h prior to use. 6,6'-(*o*-Phenylenediseleno)-bridged bis(β-CD) (**1**), mono-6-deoxy-6-ethylenediamino-*β*-CD and adamantylamine-modified gold nanoparticles (AuNPs) were synthesized according to the reported procedures.[1, 2] Crude *N,N*-dimethylformamide (DMF) was stirring in calcium hydride for three days and then distilled under reduced pressure prior to use. Milli-Q water (18.2 MΩ) was prepared using Milli-Q Synthesis System (BARNSTEAD EASYPURE Ⅱ, Thermo Scientific, USA). All other reagents and solvents were of analytical grade and used as received.

Instruments. ^{1}H NMR spectra were recorded on a Bruker AV400 instrument. Mass spectra were performed on a Thermofinnigan LCD Advantage LC-MS. Elemental analyses were performed on a Perkin-Elmer-2400C instrument. UV/Vis spectra were recorded in a conventional quartz cell (light path 10 mm) by using a Shimadzu UV-3600 spectrophotometer equipped with a PTC-3348WI temperature controller to keep the temperature at 25℃. Steady-state fluorescence spectra were recorded in a conventional quartz cell (light path 10mm) on a Varian Cary Eclipse equipped with a Varian Cary single–cell peltier accessory to control temperature (DOX: λ_{ex} = 490 nm; bandwidth (ex), 20 nm; bandwidth (em), 10 nm; CPT: λ_{ex} = 370 nm; bandwidth (ex), 5 nm; bandwidth (em), 5 nm; CPT-11: λ_{ex} = 368 nm; bandwidth (ex), 2.5 nm; bandwidth (em), 5 nm; TPT: λ_{ex} = 383 nm; bandwidth (ex), 5 nm; bandwidth (em), 5 nm). TEM images were obtained on a JEOL JEM-2010FEF high-resolution transmission electron microscope with an accelerating voltage of 200 kV. The samples were prepared by placing a drop of solution onto copper grid and air-dried. In TEM image, eighty AuNPs were

randomly selected to measure the average diameter. For AFM measurements, a drop of sample solution was dropped onto newly clipped mica and then air-dried, then examined by using an atomic force microscope (Veeco Company, Multimode, Nano IIIa) in tapping mode in air at room temperature. The sample solutions for DLS experiments were prepared by filtering each solution through a 450 nm syringe-driven filter (JET BIOFIL) into a clean scintillation vial. The samples were examined on a laser light scattering spectrometer (BI-200SM, BROOKHAVEN Company) equipped with a digital correlator (BI-9000AT) at λ = 636 nm at 25 °C. All DLS measurements were performed at the scattering angle of 90°. The zeta potential was recorded on ZETAPALS/BI-200SM (BROOKHAVEN Company) at 25.5 °C. The amount of gold was determined using an X7 Series ICP-MS (Thermo Electron Corporation, U.S.A.).

Methods. All the data of cellular viabilities in cytotoxicity experiments were expressed as mean ± standard deviation, and the statistical analysis of the data was carried out using the Student's t test. Differences were considered statistically significant if the P value was < 0.01.

Synthesis of biotin-modified β-CD (2). Biotin (80.99 mg, 1.89 mmol) and 1-hydroxybenzotrinzole (HOBt; 44.77 mg, 1.89 mmol) were dissolved in dry DMF (10 mL) and stirred in an ice-bath for 0.5 h under N_2. A solution of mono-6-deoxy- 6-ethylenediamino-*β*-CD (300 mg, 1 mmol) and *N,N'*-dicyclohexylcarbodiimide (DCC; 50.62 mg, 1.45 mmol) in dry DMF (10 mL) was then added dropwise slowly. The reaction mixture was stirred at 0°C under an N_2 atmosphere for 24h, and then stirred at room temperature for another 24 h. The mixture was filtered, and the filtrate was dried under reduced pressure to remove the solvent. The residue was dissolved in a little amount of hot water and poured into acetone (300 mL).

The precipitate was collected by filtration and was purified by column chromatography (silica gel) using propanol / H_2O / 25% ammonia (v:v:v = 6:3:1) as eluent to give **2** as a white solid with a yield of 30 % (R_f = 0.2). ^{1}H NMR (400 MHz, D_2O, TMS): δ = 1.91-2.27 (H of biotin, H of –CH_2–), 1.91-2.27 (H of biotin, H of –NH_2–), 3.09-2.45 (m, 6H, H of biotin, H of –CH_2–), 3.15-4.01 (m, 42 H, H of C-3, C-5, C-6, C-2, C-4 of β-CD), 4.31 (s, 1H, H of biotin), 4.87-5.12 (m, 7H, H of C-1 of β-CD); ESI-MS: *m/z*: 1403.6 $[M+H]^+$.

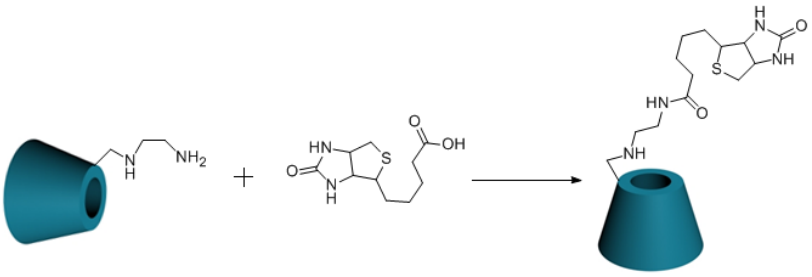

Figure S1. Synthesis scheme of **BioCD**.

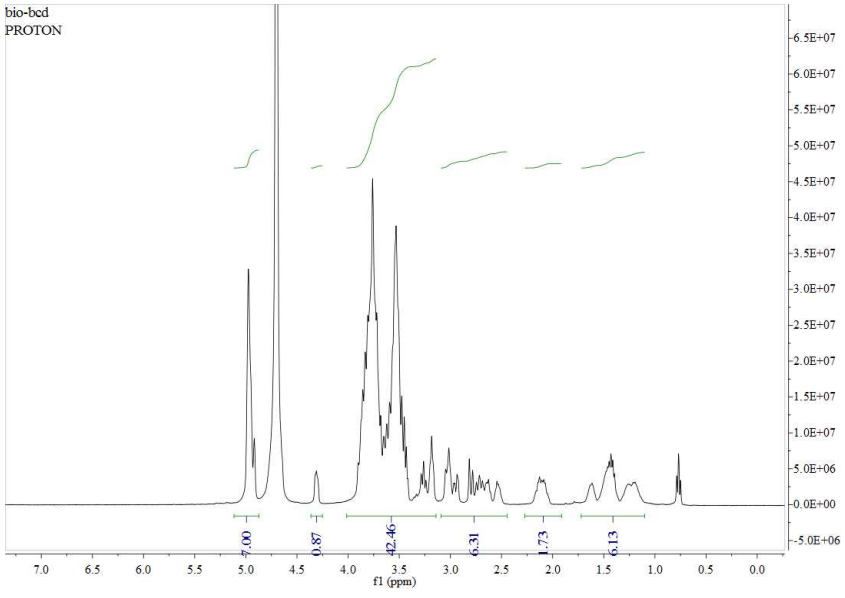

Figure S2. ^{1}H NMR (400 MHz) spectrum of **2** in D_2O at 298.15K.

Figure S3. ESI mass spectrum of **2**.

Synthesis of CD/gold nanoparticles (CD-AuNPs). 20 mg of **1** and 3 mg of **2** was dissolved in mlli-Q water (8 mL). Then 3 mg of adamantylamine-modified gold nanoparticles was added to the mixture to give **CD-AuNPs** in situ.

Drug loading on CD-AuNPs. CD-AuNPs containing 20 mg of **BisCD**, 3 mg of **BioCD** and 3 mg of AuNPs in 8 mL of mlli-Q water was freshly prepared. To the resulting solution was slowly added a PTX aqueous (2.5 mg mL^{-1}) solution containing 3% DMSO, and stirred for 24 h at room temperature in darkness. The product (PTX@CD-AuNPs) was collected by centrifugation, washing with mlli-Q water for four times, and dried in vacuo. The resulted product was redispersed in mlli-Q water prior to further characterization. The free PTX present in the supernatant, the product PTX@CD-AuNPs and CD-AuNPs (control) were determined by measurements of their UV absorbance at 227 nm to estimate the encapsulation efficiency and the percentage loading of drug on CD-AuNPs by following formula:

$$\text{Encapsulation efficiency (\%)} = 100\ m_{\text{PTX in nanoparticles}} / m_{\text{total PTX}}$$

$$\text{Drug loading efficiency (\%)} = 100\ m_{\text{PTX in nanoparticles}} / m_{\text{nanoparticles}}$$

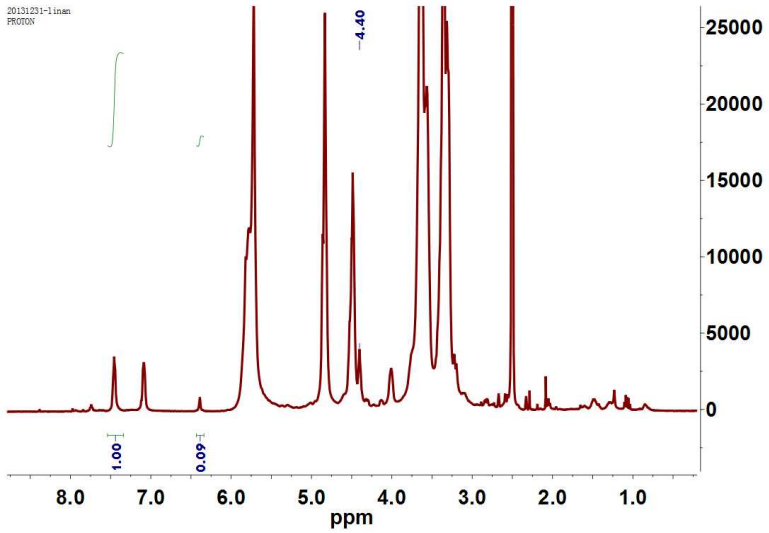

Figure S4. ^{1}H NMR of CD-AuNPs (400 MHz, 298.15K, D_2O)

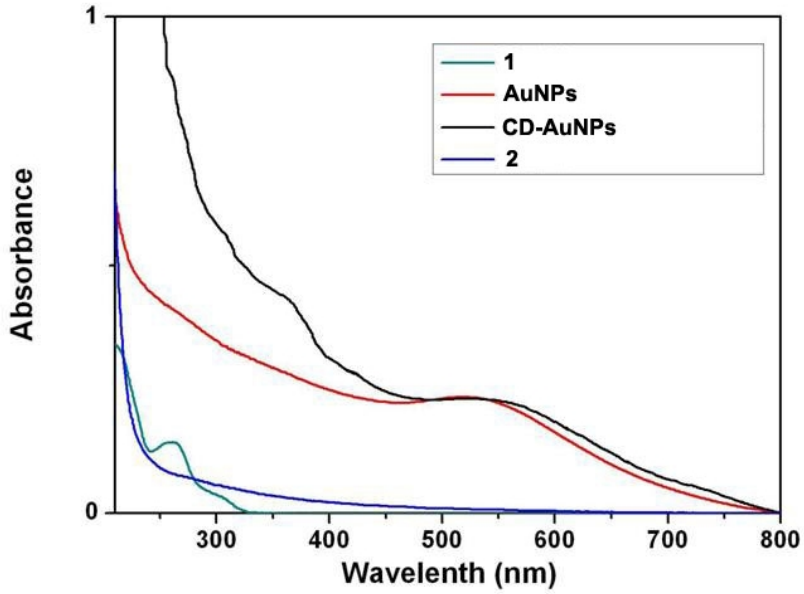

Figure S5. UV-Vis spectra of **1**, AuNPs , **2** and CD-AuNPs.

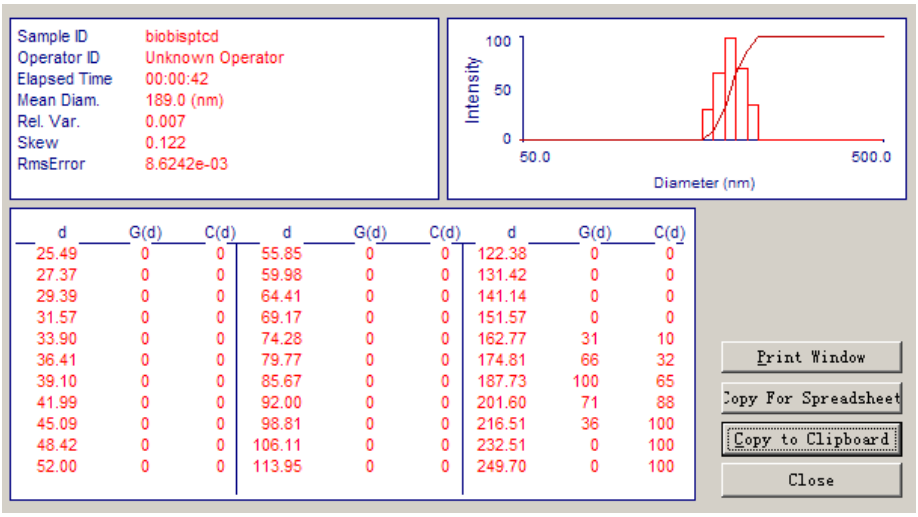

Figure S6. DLS data of CD-AuNPs.

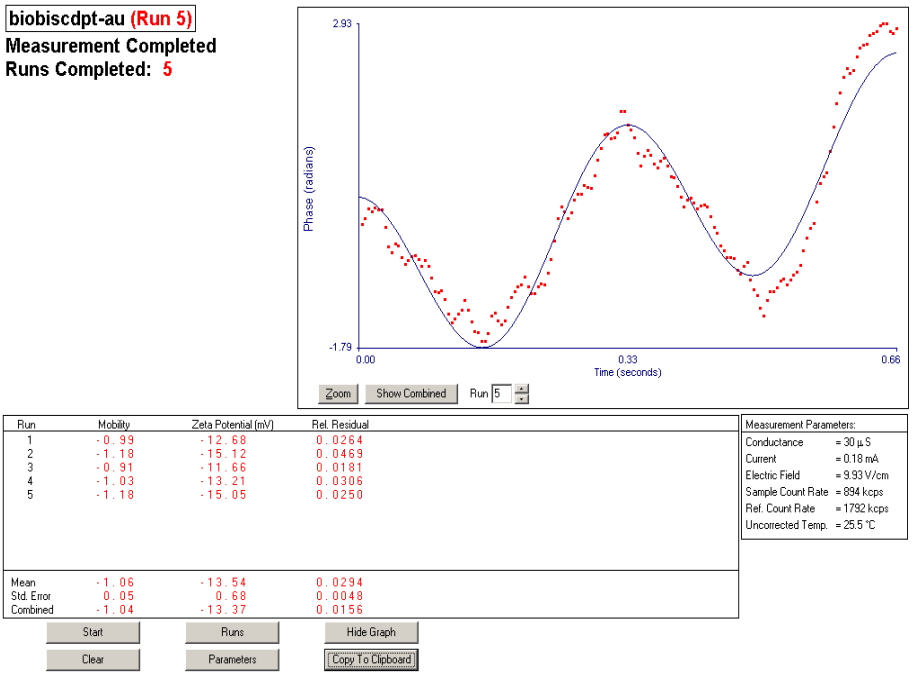

Figure S7. Zeta potential of CD- AuNPs.

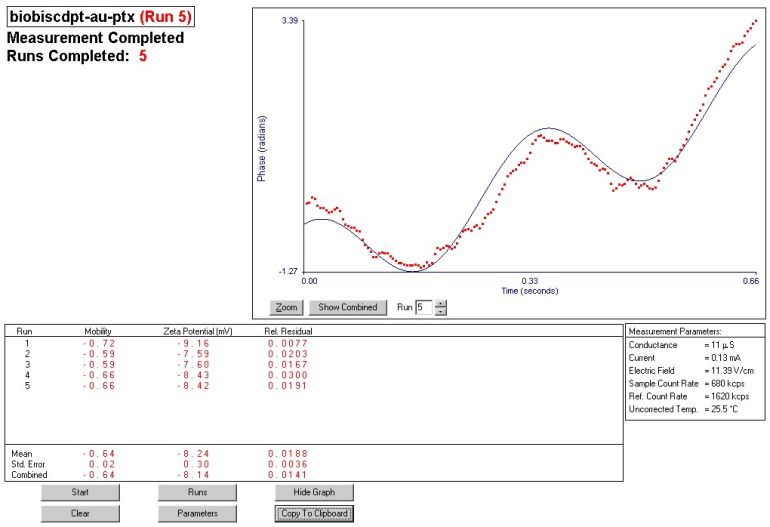

Figure S8. Zeta potential of PTX@CD-AuNPs.

In *vitro* drug release. The release of PTX from PTX@CD-AuNPs was carried out using the dialysis method at 37 °C and was compared with free PTX dissolved in phosphate buffer solution (pH 5.7 and 7.2, I = 0.01 M). Each sample solution (10 mL, [PTX] = 0.041 mg mL^{-1}) was placed in a dialysis membrane (M_W cut off = 8000-14000) and tightly sealed. Each dialysis bag was immersed in a beaker containing 400 mL of phosphate buffer with gentle stirring. At various time intervals, samples (3 mL) were taken out from buffer solution outside the dialysis bag and replaced with an equal volume of fresh buffer solution. The amount of PTX released was analyzed by measuring the absorption at 227 nm.

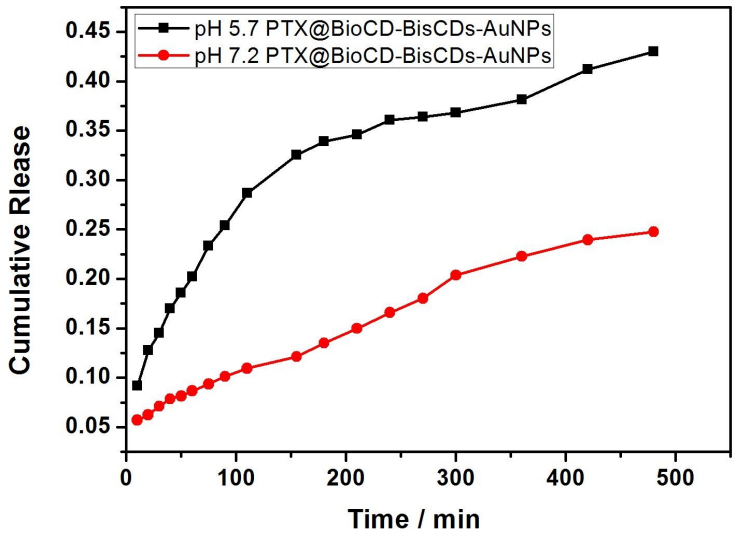

Figure S9. Release profiles of PTX from PTX@CD-AuNPs in phosphate buffer solution (*I* = 0.01 M) at pH 5.7 and 7.2 at 37 °C.

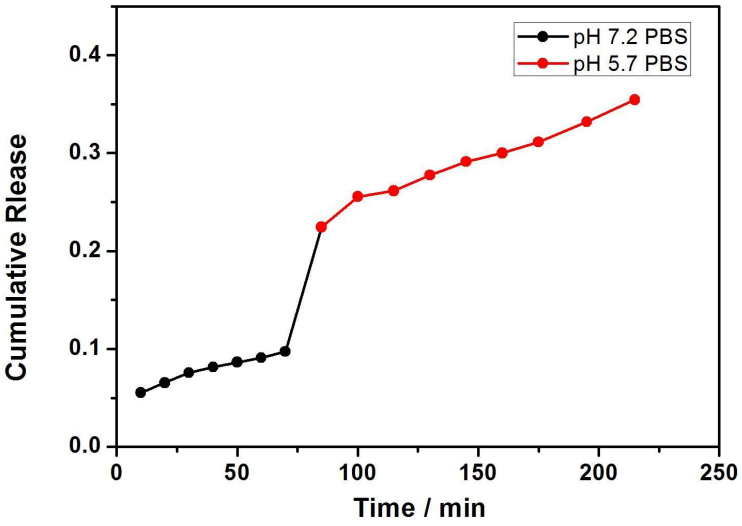

Figure S10. Sequential release profiles of PTX from PTX@CD-AuNPs in phosphate buffer solution (*I* = 0.01 M) from pH 5.7 to 7.2 at 37 °C.

Cytotoxicity experiment. SKOV-3 human ovarian cancer cells and NIH3T3 mouse embryo fibroblasts were incubated in Dulbecco's modified Eagle's medium (DMEM) supplemented

with 10% fetal bovine serum (FBS) in 96-well plates (2×10^4 cells mL^{-1}, 100 μL per well) at 37°C under a humidified atmosphere with CO_2 (5%) for 24 h. The cells were incubated with PTX, CD-AuNPs, and PTX@CD-AuNPs, respectively ([PTX] = 0.085 μg mL^{-1}, [AuNPs] = 0.07 μg mL^{-1}, and [CD] = 0.9 μg mL^{-1}). After incubation for 24 and 48 h, the cells were washed with PBS, and then MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltertrazolium bromide) (50 μL, 4 mg mL^{-1}) was added into each well. The cells were continued to grow for an additional 4 h, then the medium was removed, and 100 μL DMSO was added into each well. After 30 minutes, the absorbance of dissolved formazan was measured using a Bio-Rad microplate reader at 540 nm. All experiments were performed in triplicate, and all the data were presented as the averaged results and standard deviation.

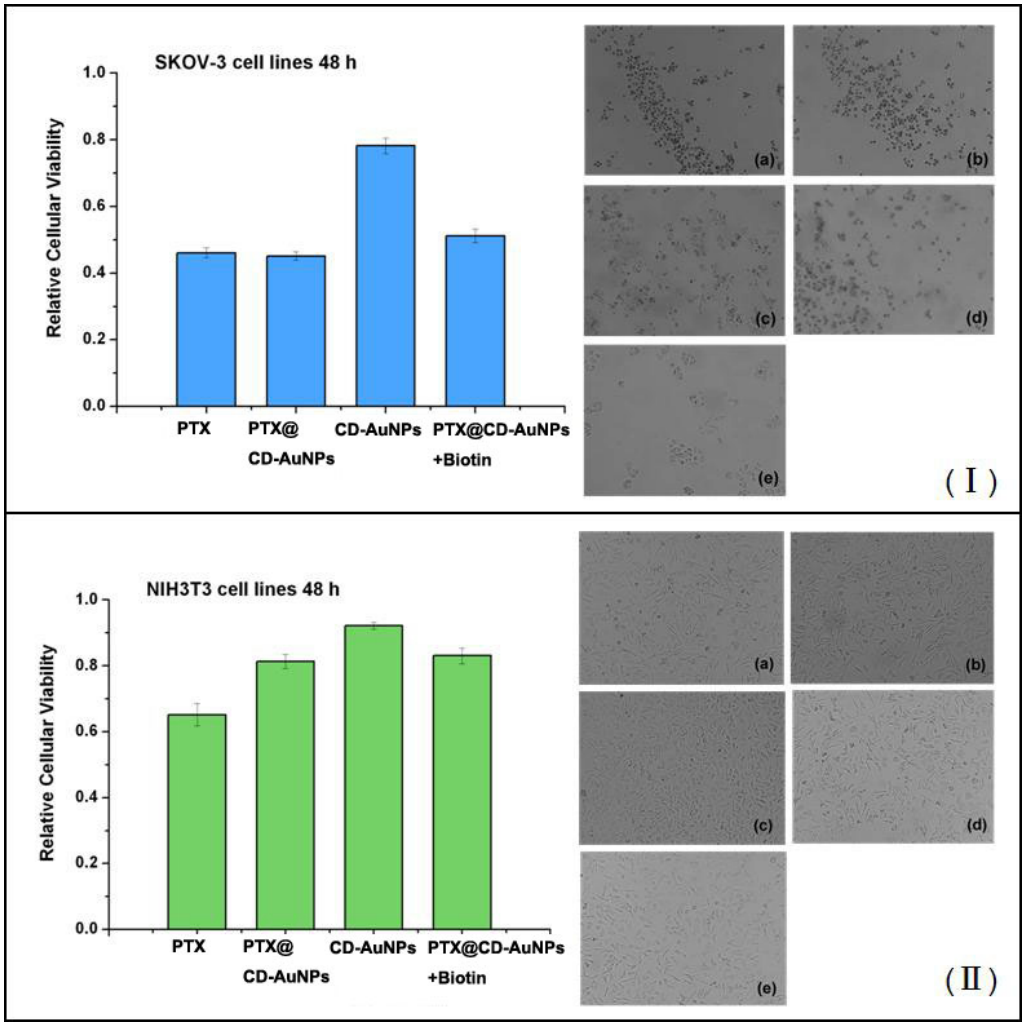

Figure S11. Cytotoxicity experiments *in vitro* in 48 h. Relative cellular viability of SKOV-3 cell lines (I left) and NIH3T3 cell lines (II left) 24 h after treatment with PTX, PTX@CD-AuNPs, CD-AuNPs, and PTX@CD-AuNPs with excess amount of biotin. Photos of SKPV-3 cell lines (I right) and NIH3T3 cell lines (Ⅱ right) treated with (A) PTX, (B) PTX@CD-AuNPs (C) CD-AuNPs, (D) PTX@CD-AuNPs with excess amount of biotin, (E) blank are shown.

Reference

1. Liu, Y.; Li, B.; You, C.-C.; Wada, T.; Inoue, Y. *J. Org. Chem*. **2001**, *66*, 225-232.

2. Li, N.; Chen, Y.; Zhang, Y.-M.; Yang, Y.; Su, Y.; Chen, J.-T.; Liu, Y. *Sci. Rep.* **2014**, *4*, 4164.