Supporting Information

Cyclodextrin-Covered Gold Nanoparticles for Targeted Delivery of an Anti-Cancer Drug

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Experimental section

Synthesis

Synthesis of per-6-thio-β-cyclodextrin (SH-CD). The perthiolated β-CD was prepared following a reference procedure.[1,2]

^{1}H NMR (400MHz, D_2O) δ 2.16 (t, J=6.0Hz, 7H, -SH), 2.79 (m, 7H, H-6a), 3.21 (br d, J=14Hz, H-6b), 3.36-3.40 (m, 14H, H-2, H-4), 3.60 (t, J=9.0Hz, 7H, H-3), 3.68 (t, J=8.0H, 7H, H-1), 5.83 (s, 7H, 3-OH), 5.97 (d, J=6.0Hz, 7H, 2-OH); MS (FAB-MS) calcd for $C_{42}H_{70}O_{28}S_7$ 1247.40, found 1251 [M+4H]+.

Synthesis of NHS-PEG-SH. A THF solution (1 mL) of N-hydroxysuccinimide (12.5 mg, 0.11 mmol) and triethylamine (0.015 mL, 0.11 mmol) was added to a THF solution (5 mL) of α-carboxy-ω-hydroxy - poly(ethylene glycol) (0.1 g), 1,3-diisopropylcarbodiimide (0.022 g, 0.11 mmol), and 4-(dimethylamino)pyridine. After stirring for 12 h at 45 °C, the precipitated urea was filtered off. The purified product was obtained by precipitation from THF into diethyl ether (yield 0.08 g, 80 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.4 (b, 50H, -CO-CH_2-CH_2-*CH_2*-CH_2-CH_2-O-), 1.6 (b, 95H, -CO-CH_2-*CH_2*-CH_2-*CH_2*-CH_2-O-), 2.25 (b, 50H, -CO-C*H_2*-CH_2-), 2.83 (b, 4H, -CO-C*H_2*-C*H_2*-CO-), 3.6 (m, 200H, -C*H_2*-C*H_2*-O-), 4.1 (b, 46H, -CO-CH_2-CH_2-*CH_2*-O-).

Synthesis of Rho-CD. Rho-CD was prepared by a coupling reaction between rhodamine B and SH-CD. SH-CD (300 mg, 240 μmol) and rhodamine B (576 mg, 2.1 mmol) were added to a dry DMF solution (20 mL) of p-toluenesulfonic acid (16 mg, 84 mmol), and the reaction was allowed to reflux for 10 hours. The mixture was concentrated under reduced pressure and poured into cold acetone (100 mL) to yield Rho-CD. (Yield 0.17 g).

^{1}H NMR (400MHz, DMSO-d_6) δ 0.86-1.01 (m, C*H_3*-C*H_2*-N-), 2.16 (t, SH), 2.80 (m, H-6a), 3.19-4.03 (m, H-2, H-3, H-4, H-5, H-6b), 4.99 (s, H-1), 5.91-6.00 (m, OH- of CD), 6.98-8.22 (m, Rhodamine); MS (FAB-MS) calcd for $C_{70}H_{99}ClN_2O_{30}S_7$ 1706.4, found 1748.

Preparation of gold nanoparticles (AuNP-0). Citrate-stabilized gold nanoparticles (**AuNP-0**) were prepared by following a modified manner of literature method. All glassware was cleaned in aqua regia (3 parts HCl, 1 part HNO_3), rinsed with doubly distilled H_2O, and then oven dried prior to use. An aqueous

solution of 0.02 % $HAuCl_4$ (800 mL) was brought to a reflux while stirring, and then 15 mL of 2 % trisodium citrate solution was added quickly, which resulted in a change in solution color from pale yellow to deep red. After the color change, the solution was refluxed for an additional 15 min, allowed to cool to room temperature, and subsequently filtered through a 0.45 μm cellulose acetate filter.

The concentration of gold nanoparticles was calculated based on extinction spectra by using calculated value of the extinction coefficient for citrate-stabilized gold nanoparticles. The diameters of the AuNPs were determined by TEM analysis.

Preparation of AuNP-1. α-Methoxy-ω-mercapto-poly(ethylene glycol) (Mw 2,000, 0.13 g, 65 μmol) and per-6-thio-β-cyclodextrin (0.1 g, 0.08 mmol) were dissolved in 5 mL of DMF, and then the solution was added into 625 mL of 30 nm diameter Au nanoparticle solution (0.54 mM). After stirring for 3 days, the solution was purified by centrifugation and washing with water.

Preparation of AuNP-1.5. α-Methoxy-ω-mercapto-poly(ethylene glycol) (MW 2,000, 0.12 g, 60 μmol), α-succinimidyl propionate-ω-lipoic acid-poly(ethylene glycol) (Mn 5,350, 0.035 g, 6.5 μmol) and per-6-thio-β-cyclodextrin (0.3 g, 0.24 mmol) were dissolved in 5 mL of DMF, and then the mixture was added into 625 mL of 30 nm diameter Au nanoparticle solution (0.54 mM). After stirring for 3 days, the solution was purified by centrifugation and washing with water.

Preparation of AuNP-2. Anti-EGFR (12.4 mg, 72.9 nmol) was added to a PBS solution (0.4 mL, pH=7.4) of AuNP-1.5 (1.7 mg) and stirred for 48 h at room temperature. The reaction mixture was then dialyzed against PBS solution for 48 h. The conjugation of anti-EGFR on the surface of AuNP-2 was characterized by using BCA protein assay and dot blot.

Dot blot assay. Nitrocellulose membrane was labeled for protein elution fractions and placed on the top of wet Whatman paper for keeping moist. Samples (2 μL each) were loaded from each AuNP carrier fraction on the membrane, which was then allowed to dry. The membrane was incubated in blocking solution of 5 % skim milk for 1 h. After washing twice with TBST, the membrane was immersed into the secondary antibody (anti-mouse immunoglobulin G; Amersham Pharmacia Biotech) solution, followed

by 1 h shaking at room temperature. The membrane was then washed four times for 15 min each with TBST buffer. Specific antigen-antibody complexes were detected using the ECL Western blotting detection system (Amersham Pharmacia Biotech). The excess amount of ECL substrate solution was drained from the membrane, which was then wrapped and exposed to X-ray film to detect anti-EGFR.

Thermogravimetric analysis (TGA). TGA analysis was performed on a TGA Q50. Samples were placed in platinum sample pans and heated under an argon atmosphere at a rate of 20 °C / min to 100 °C and held for 30 min to completely remove residual solvent. Samples were then heated to 700°C at a rate of 20°C / min.

Determination of the number of ligands in the AuNP carriers. TGA for **AuNP-1**: Au nanoparticle, 95.53 %; ligands (mPEG-SH and SH-CD), 4.47 %. TEM; average diameter (D) = 27 nm. The approximate number of gold atoms in a cluster; $N_{Au} = (59\ nm^{-3})(\pi /6)D^3 = 608,053$. (The density of bulk face-centered cubic (fcc) Au is 59 atoms/nm^3). The proportion of ligands is given by the ^{1}H NMR spectrum (**AuNP-1**; SH-CD : mPEG-SH = 0.35 : 1). The approximate number of SH-CD per AnNP-1 is $n_{SH-CD} = 800$; $n_{mPEG-SH} = 2250$.

TGA for **AuNP-1.5**: Au nanoparticle, 95.51%; ligands (mPEG-SH, NHS-PEG-SH and SH-CD), 4.49%. TEM; average diameter (D) = 27 nm. The approximate number of gold atoms in a cluster; $N_{Au} = (59\ nm^{-3})(\pi /6)D^3 = 608,053$. (The density of bulk face-centered cubic (fcc) Au is 59 atoms/nm^3). The proportion of ligands is given by the ^{1}H NMR spectrum (**AuNP-1.5**; SH-CD : mPEG-SH: NHS-PEG-SH = 0.438 : 1: 0.1). The approximate number of SH-CD per AnNP-1 is $n_{SH-CD} = 800$; $n_{mPEG-SH} = 1823$; $n_{NHS-PEG-SH} = 180$.

Determination of drug-loading content. The drug-loading contents were measured by reverse titrations. The mixture solution of β-lapachone and Au nanocarrier was sonicated for 1 minute and an excess amount of drugs were removed by ultracentrifugation and washing with deionized water. The drug loading content was determined by measuring the UV-vis absorbance of the supernatant at 330 nm.

Dynamic light scattering. Dynamic light scattering measurements were performed using a Brookhaven BI-200SM goniometer and BI-9000AT digital autocorrelator. All the measurements were carried out at

room temperature. The sample solutions were purified by passing through a Millipore 0.45 μm filter. The scattered light of a vertically polarized He-Ne laser (632.8 nm) was measured at an angle of 90° and was collected on an autocorrelator. The hydrodynamic diameters (d) of vesicles were calculated by using the Stokes-Einstein equation $d = k_B T/3\pi\eta D$, where k_B is the Boltzmann constant; T is the absolute temperature; η is the solvent viscosity; and D is the diffusion coefficient. The polydispersity factor of micelles, represented as μ_2/Γ^2, where μ_2 is the second cumulant of the decay function and Γ is the average characteristic line width, was calculated from the cumulant method.[3,4] CONTIN algorithms were used in the Laplace inversion of the autocorrelation function to obtain size distributions.[5]

Transmission electron microscopy experiments. Transmission electron microscopy (TEM) was performed using a Philips CM 200, operated at an acceleration voltage of 120 kV. For the preparation of dispersed samples in water, a drop of sample solution (50 mg/L) was placed onto a carbon-coated copper grid. About 2 min after deposition, the grid was tapped with filter paper, followed by air-drying. Negative staining was performed by using a droplet of a 2 wt % uranyl acetate solution. The samples were air-dried before measurement.

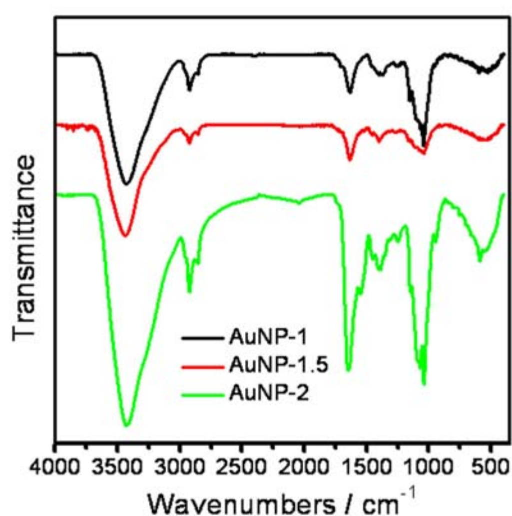

Figure S1. FT-IR spectra of **AuNP-1**, **AuNP-1.5**, and **AuNP-2**.

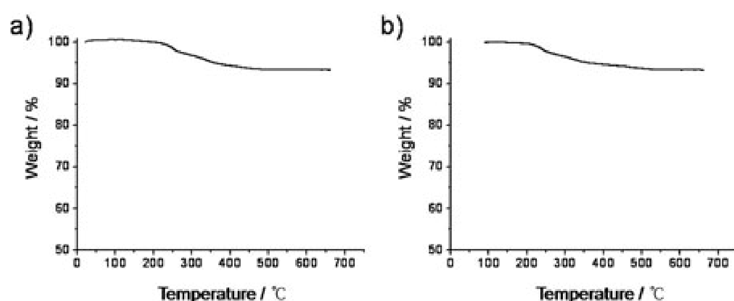

Figure S2. TGA data of (a) **AuNP-1** and (b) **AuNP-1.5**, respectively.

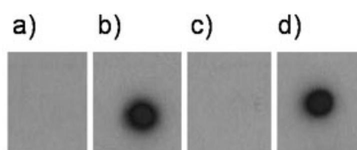

Figure S3. Dot blot analysis of a) **AuNP-1**, b) **AuNP-2**, c) **AuNP-1/lap**, and d) **AuNP-2/lap**, respectively.

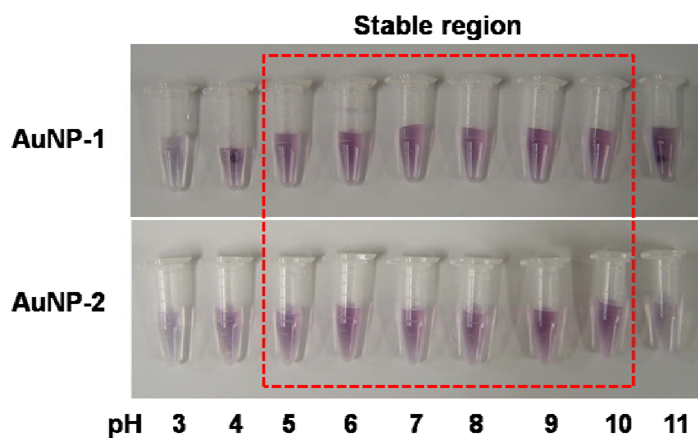

Figure S4. Colloidal stability test of **AuNP-1** and **AuNP-2** against pH.

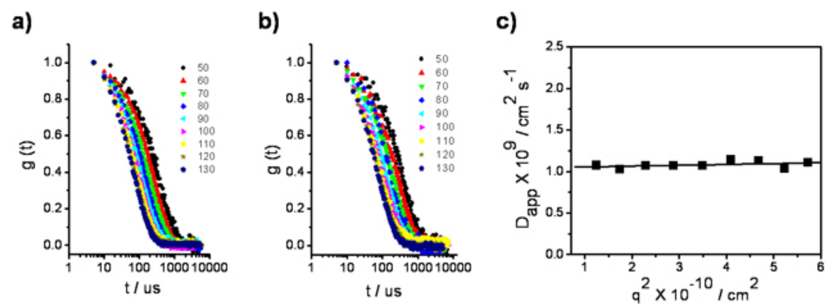

Figure S5. Autocorrelation functions of the (a) **AuNP-1/lap** and (b) **AuNP-2/lap** at different scattering angles. (c) Angular dependence of **AuNP-1/lap** (T = 25 ℃).

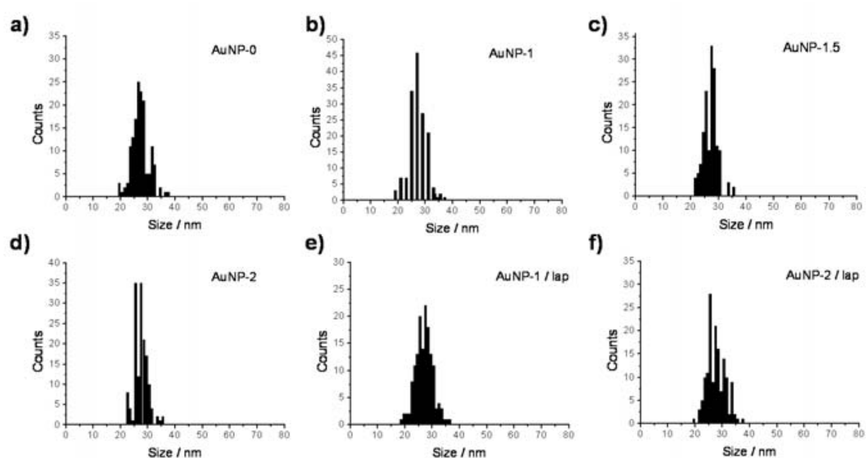

Figure S6. Size distribution histograms of (a) **AuNP-0**, (b) **AuNP-1**, (c) **AuNP-1.5**, (d) **AuNP-2**, (e) **AuNP-1/lap**, and (f) **AuNP-2/lap**, respectively.

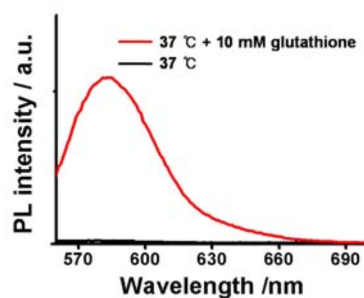

Figure S7. Fluorescence spectra of **RhoCD-AuNP-2** before (black) and after treatment with glutathione (red).

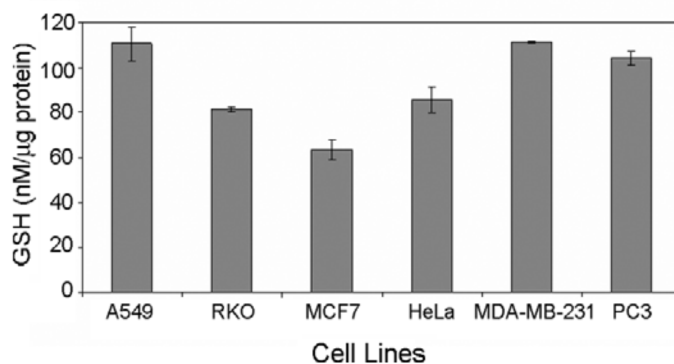

Figure S8. Glutathione concentration in different cell lines.

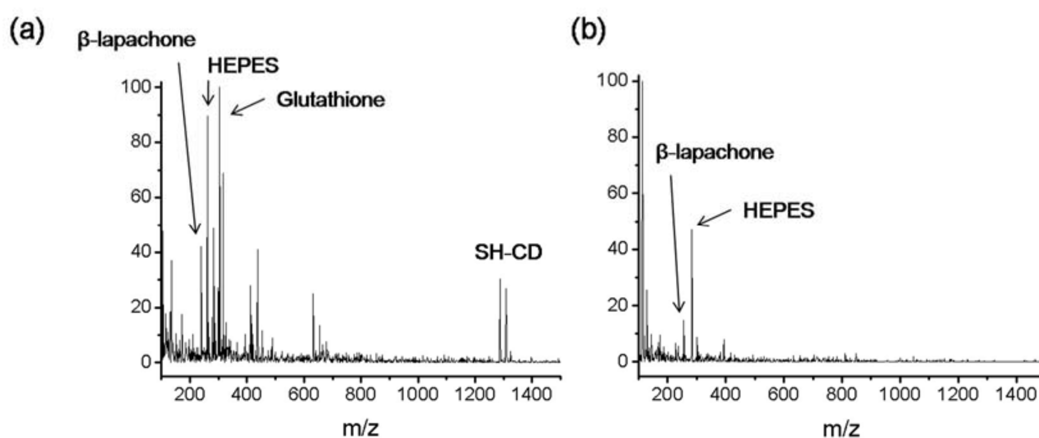

Figure S9. ESI-MS data of released samples from **AuNP-1/lap** in (a) glutathione solution (HEPES buffer, pH 7.4) and (b) HEPES buffer solution (pH 7.4) at 37˚C.

References

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