Supporting information

Intracellular host-guest assembly of gold nanoparticles triggered by glutathione

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Experimental section

Materials

Gold nanoparticles (AuNPs) with size of 16 nm were synthesized according to our previous studies^[1]. Mono-6-thio- β -cyclodextrin (β -CD-SH) was prepared by published procedure with a slight modification^[2]. Ferrocene (Fc) capped poly(ethylene glycol) (Fc-PEG2k-Fc)^[3]and mercaptopoly(ethylene glycol) (HS-PEG2k, Mw = 2000)^[4] were obtained according to literatures, respectively. *D*, *L*-dithiothreitol (DTT) was purchased from Aladdin and used as received. Glutathione reduced ethyl ester (GSH-OEt) and buthionine sulfoximine (BSO) were supplied by Sigma-Aldrich and used as received.

Modification of AuNPs simultaneously by β -CD-SH and PEG-SH (GNPs)

Specifically, a mixed thiol aqueous solution (0.025 mM, 1 mL) that containing β -CD-SH and PEG-SH at the mole ratio of 1:1 was added into the original citrate-coated 16 nm AuNP solution (50 mL). In order to facilitate ligand exchange reaction, the pH of the solution was adjusted to pH ~9 by 1 M NaOH. After stirring at room temperature for 24 h, the modified AuNPs were purified by centrifuging twice at 16 000 rpm for 10 min (GNPs). Then the GNPs were redispersed in water and adjusted the pH of the resultant solution to pH 7.4 by the phosphate buffer solution (10 mM PB, pH 7.4).

Synthesis of ferrocenium cation capped PEG (Fc⁺-PEG-Fc⁺)

Oxidation reaction of Fc-PEG-Fc by anhydrous ferric trichloride was following published procedure^[5]. Particularly, Fc-PEG-Fc was dissolved in 9 mL of CH_2Cl_2 , then FeCl₃ (0.1 mmol) in 1 mL of CH_3CN was added dropwise. After stirring for 24 h, Fc⁺-PEG-Fc⁺ was obtained by evaporating to dryness.

In vitro study of assembly behavior between GNPs and Fc⁺-PEG-Fc⁺

For the purpose of comparison, the volume of GNPs added into four flasks was the same (80 μ L). Then, 400 μ L of Fc⁺-PEG-Fc⁺ (10 mg mL⁻¹) was added into flask b and c. 25 μ L of DTT was also added into flask c and d. Finally, these four flasks were diluted by a certain volume of water. The resultant volume of the solution was 1 mL. Photos were taken at the very beginning and after 3 hours' incubation.



NIR Photothermal Conversion Efficiency

The four flasks used above were further used for this experiment. The temperature rise of each sample was recorded in intervals during 5 min irradiation by 13.8 W/cm² 808 nm continuous wave (CW) laser (MDL-N-808 nm, Changchun New Industries Optoelectronics Tech. Co., Ltd., ~6 mm diameter spot-size).

Cell culture

Human hepatocellular carcinoma cells (HepG2 cells) were cultured with high-glucose Dulbecco's modified Eagle medium (DMEM). The cell growth media was supplemented with 10% fetal bovine serum, 100 U mL⁻¹ penicillin, and 100 mg mL⁻¹ streptomycin, and cultured at 37 °C in a 5% CO₂ humidified environment.

Cytotoxicity assays

Cytotoxicity was performed by staining of fluorescein diacetate (FDA) or the standard MTT assay. To determine cell viability, the HepG2 cells were plated at a density of 3×10^4 cells per well in a 96-well plate and cultured for 24 h. Then the medium was replaced with fresh medium containing GNPs (40 µL), 20 µL of Fc⁺-PEG-Fc⁺ (10 mg mL⁻¹) or GNPs/Fc⁺-PEG-Fc⁺ (40 µL/20 µL). After incubation for 5 h, the sample was irradiated by a 12.7 W/cm² 808 nm NIR CW laser (Hi-Tech Optoelectronics Co., Ltd.) with a 1 mm focused spot size for 1 min. After irradiation, the media was replaced by DMEM medium. As for the staining of FDA, The cells were stained with FDA to determine the cell viability, as live cells could give out green fluorescence from FDA with the inside esterase, whereas dead cells could not give out fluorescence. As for MTT assay, 20 µL MTT (5 mg mL⁻¹) was added to each well and the cells were further cultured at 37 °C for 4 h. The dark blue formazan crystals generated by the mitochondria dehydrogenase in the live cells were dissolved with 150 µL DMSO to measure the absorbance at 490 nm by a microplate reader (MODEL 550, Bio Rad). Experiments were performed in quintic.

Verification of GSH's role in intracellular assembly

The experiment was carried out in a similar way to that of MTT assay. HepG2 cells were plated at a density of 3×10^4 cells per well in a 96-well plate and cultured for 24 h. Before adding the samples,

cells were treated with BSO for 12 h or GSH-OEt for 2 h. Untreated cells were used as control. Then the medium was replaced with fresh medium containing GNPs (40 μ L), 20 μ L of Fc⁺-PEG-Fc⁺ (10 mg mL⁻¹) or GNPs/Fc⁺-PEG-Fc⁺ (40 μ L/20 μ L). After incubation for 5 h, the sample was irradiated by a 12.7 W/cm² 808 nm NIR CW laser (Hi-Tech Optoelectronics Co., Ltd.) with a 1 mm focused spot size for 1 min. After irradiation, the media was replaced by pH 7.4 DMEM medium. 20 μ L MTT (5 mg mL⁻¹) was added to each well and the cells were further cultured at 37 °C for 4 h. The dark blue formazan crystals generated by the mitochondria dehydrogenase in the live cells were dissolved with 150 μ L DMSO to measure the absorbance at 490 nm by a microplate reader (MODEL 550, Bio Rad). Experiments were performed in quintic.

Cell slices

Cell slices were observed by JEM-1230 TEM at an accelerating voltage of 80 kV. HepG2 cells were seeded on a 24-well plate at a density of 3×10^5 cells per well and cultured for 24 h. Then, GNPs (400 µL) and GNPs/Fc⁺-PEG-Fc⁺ (400 µL/200 µL) was added after the wells were washed with PBS and replaced with fresh media. After incubation for 5 h, the cells were washed five times with PBS and trypsizined, centrifuged, and then fixed with 2.5% glutaraldehyde. After 2 h fixation at 4 °C, the samples were washed with phosphate-buffered saline (0.1 M, pH = 7.0) three times. Then the samples were fixed with 1% perosmic oxide for 2 h at 4 °C. After being washed by water, the samples were dehydrated in an alcohol series, embedded, and sliced with a thickness of 50 to 70 nm.

Retention test of GNPs in cancer cells

Retention test of GNPs in cancer cells was determined by ICP-MS quantitatively. HepG2 cells were

incubated into 24 well plates at a density of 3×10^5 cells per well in DMEM medium for 24 h. Then, GNPs (400 µL) and GNPs/Fc⁺-PEG-Fc⁺ (400 µL/200 µL) was added after the wells were washed with PBS and replaced with fresh media. After incubation for 5 h, the cells were further washed by PBS and replaced with fresh media to culture for 2, 4 and 6 h. Cells were washed five times with PBS and then treated with aqua regia (HCl/HNO₃ = 1:3, V:V) for 2 h. The treated solution was diluted for 20 times to measure Au concentration by ICP-MS (Thermo Elemental Corporation of USA, X Series II).

Cell uptake of GNPs by HepG2 cells

Cell uptake of GNPs by HepG2 cells was also determined by ICP-MS. HepG2 cells were incubated into 24 well plates at a density of 3×10^5 cells per well in DMEM medium for 24 h. Then, GNPs (400 µL) and GNPs/Fc⁺-PEG-Fc⁺ (400 µL/200 µL) was added after the wells were washed with PBS and replaced with fresh media. After incubation for different time, cells were washed five times with PBS and then treated with aqua regia (HCl/HNO₃ = 1:3, V:V) for 2 h. The treated solution was diluted for 20 times to measure Au concentration by ICP-MS (Thermo Elemental Corporation of USA, X Series II).

Cell apoptosis

HepG2 cells were seeded on a 24-well plate at a density of 3×10^5 cells per well and cultured for 24 h. Then, GNPs (400 µL) and GNPs/Fc⁺-PEG-Fc⁺ (400 µL/200 µL) was added after the wells were washed with PBS and replaced with fresh media. After incubation for 5 h, cells were treated with trypsin and centrifuged for 5 min at 1000 rpm after being washed three times with PBS. Then the cells were suspended in 500 µL binding buffer. Then 5 µL Annexin V-FITC and 5 µL PI were

added and incubated with the cells for 15 min in the dark. Finally, the stained cells were analyzed using a FACScan flow cytometer.

Characterizations

The ¹H NMR spectra were recorded on a Bruker DMX500 spectrometer operating at 500 MHz using deuterated CDCl₃ or DMSO- d_6 as the solvent. The sizes of samples were measured using dynamic light scattering (DLS). Measurements were performed using Zetasizer Nano-ZS from Malvern Instruments equipped with a He-Ne laser at the wavelength of 633 nm with an angle of 173° (25 °C). The sizes and morphologies of the samples were also characterized by HT7700 transmission electron microscopy (TEM) at an accelerating voltage of 100 kV, whereby a carbon-coated copper EM grid (230 mesh) was immersed into the sample solution for a while and dried at room temperature at atmospheric pressure. UV-visible spectra were obtained using a UV–vis Shimadzu UV-2505 spectrophotometer.



Scheme S1 Synthetic route of β -CD-SH.



Figure S1. ¹H NMR spectrum of β -CD-SH in DMSO- d_6 .



Figure S2. Mass spectrum of β -CD-SH, $[M+H]^+ = 1194.3$.



Figure S3. ¹H NMR spectrum of Fc-PEG-Fc in CDCl₃.



Figure S4. DLS plot and representative TEM image of GNPs.



Figure S5. UV spectra of Citrate-capped GNPs and GNPs.



Figure S6. UV spectra of GNPs, GNPs/Fc⁺-PEG-Fc⁺, GNPs/DTT and GNPs/Fc⁺-PEG-Fc⁺/DTT after incubation for 2 h.



Figure S7. Photothermal conversion performances of GNPs, GNPs/Fc⁺-PEG-Fc⁺, GNPs/DTT and GNPs/Fc⁺-PEG-Fc⁺/DTT after the NIR CW light irradiation.



Figure S8. DLS plot of GNPs in DMEM.



Figure S9. DLS plot of GNPs in DMEM with 10% fetal bovine serum.



Figure S10 Cell viability of HepG2 cells under 100 mW laser at 808 nm for 1 min which were incubated with GNPs/Fc⁺-PEG-Fc⁺. The cells were pretreated with 0.5 mM BSO or 10 mM GSH-

OEt. The non-pretreated cells were used as a control. *p < 0.05, **p < 0.05.



Figure S11 Uptake ratio of cells treated by GNPs/Fc⁺-PEG-Fc⁺ and GNPs. The concentration of the GNPs added was the same.



Figure S12. Apoptosis of HepG2 cells. Cells stained with annexin V-FITC and propidium iodide (PI) were characterized by flow cytometry. Untreated HepG2 cells were used as a control. Cells that were negative for both annexin V-FITC and PI staining were classified as alive, whereas cells stained positively for annexin V-FITC and negatively for PI were classified as apoptotic. Cells that stained positively for PI were classified as necrotic.



Figure S13. Photos of HepG2 cells in bright field and fluorescence field (Left represented 100 mW, right represented without light). From upper to bottom was GNPs, Fc⁺-PEG-Fc⁺, GNPs/Fc⁺-PEG-Fc⁺ and DMEM.



Figure S14. Cell viability of HepG2 cells under 100 mW laser for 1 min or not which was pretreated with Fc⁺-PEG-Fc⁺, GNPs and GNPs/Fc⁺-PEG-Fc⁺.



Figure S15. TEM images of HepG2 cells after incubation with GNPs (upper) or GNPs/Fc⁺-PEG-Fc⁺ (bottom) for 12 h.



Figure S16. UV spectra and the picture of gold nanoparticles modified with β -CD in different conditions.



DTT GSH Cys



Figure S17. Digital photos of GNPs/Fc⁺-PEG-Fc⁺ (above represents at the very beginning, below represents forming aggregation) with different kinds of reducing agents. The concentration of reducing agent was 10 mM.

References

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