Supporting Information for

Active Targeting Drug-Gold Nanorod Hybrid Nanoparticles for Amplifying Photoacoustic Signal and Enhancing Anticancer Efficacy

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EXPERIMENTAL METHODS

Equipment

Transmission electron microscopy (TEM) images were performed on a FEI Tecnai G2 F30 microscope at 200 Kv and a Hitachi HT7700 Exalens microscope at 120 Kv. UV-vis absorption
spectra were recorded by using a Shimadzu UV-2501 spectrophotometer. Fluorescence imaging of the cells were obtained by using a LSCM imaging system (Zeiss LSM 780). X-ray photoelectron spectroscopy (XPS, monochromatic Al Kα radiation)

Synthesis of Meo-PEG polyphenols with acid-labile β-thiopropionate linker

Meo-PEG-OH (0.5 g, 0.10 mmol) was dissolved in THF (10 mL) and then purged with nitrogen for 30 min to remove oxygen under an ice-water bath. After that, AC (100 μL, 1.3 mmol) and TEA (250 μL, 1.7 mmol) were added sequentially. The mixed solution was then stirred at 25 °C. After reaction for 10 h, 10 mL of mercapto acetic acid (MPA) dissolved in DMF (2 mmol) was added. The solution was then stirred for 15 h at 25 °C. The obtained polymers (Meo-PEG-AC-MPA-COOH) were washed three times using PBS (pH = 7.4) by centrifugal ultrafiltration (Millipore, molecular size cutoff of 5.0 kDa) and finally dissolved in 10 mL of water. To conjugate polyphenols, the Meo-PEG-AC-MPA-COOH and dopamine hydrochloride were added in DMF with argon bubbling for 1 h. Then TEA (34 μL) was mixed into the above solution under N₂ protection overnight. To obtain Meo-PEG polyphenols with pH-labile β-thiopropionate linker, the reaction mixture was purified by dialysis for two days, followed by lyophilisation.
Synthesis of RGD-polyphenols

The RGD-COOH conjugated polyphenols was based on the reaction between -COOH and -NH$_2$ of dopamine hydrochloride in the presence of EDC. Typically, 10 μL of EDC (50 μmol) and 100 μL of RGD-COOH (5.0 mg/mL) were added into 8.0 mL of the dopamine hydrochloride solution under magnetic stirring. The reaction was kept at room temperature for 10 h under magnetic stirring. The RGD-polyphenols were finally dissolved in PBS (pH 7.4) or saline.

Synthesis of IR780-polyphenols

To conjugate IR780 with polyphenols, the IR780 and dopamine hydrochloride were added in DMF with argon bubbling for 50 min. Then, TEA (34 μL) was mixed into the above solution under N$_2$ protection overnight. To obtain The IR780-polyphenols, the reaction mixture was purified by
dialysis for two days, followed by lyophilisation.

Scheme S3. Synthesis of PA-activating IR780-polyphenols.

**Calculation of the Photothermal Conversion Efficiency of the Hybrid NP**

The photothermal conversion efficiency ($\eta$) of the hybrid NP was calculated based on the energy balance of the system as follows:

$$\eta = \left( hS \Delta T_{\text{max}} - Q_s \right) / I \left( 1 - 10^{-A_{808}} \right)$$  \hspace{1cm} (Equation S1)

$$\tau_s = m_D C_D / hS$$  \hspace{1cm} (Equation S2)

in which $h$ is the heat-transfer coefficient, $S$ is the surface area of the container, $\Delta T\text{ max}$ is the temperature change of the vesicle solution at the maximum steady-state temperature, $I$ is the laser power, $A_{808}$ is the absorbance of the hybrid NP at 808 nm, and $Q_s$ is the heat associated with light absorption by the solvent. The variable $\tau_s$ is the sample-system time constant, and $m_D$ and $C_D$ are the mass and heat capacity (4.2 J/g) of the deionized water used as the solvent. According to equations S1 and S2, the $\eta$ value of the hybrid NP was determined to be 67.5%.

**U-87 MG Cell Culture**

U-87 MG (human glioblastoma cell line) cells were cultured in the DMEM medium
supplemented with 10 wt% of fetal bovine serum (FBS), 100 units/mL of penicillin and 100 µg/mL of streptomycin. The cells were incubated at 37 °C in a humidified atmosphere containing 5% of CO₂.

**Laser Scanning Confocal Microscopy (LSCM)**

The U87MG cells were seeded at a density of 25,000 cells per well in 8-well Lab-Tek coverglass slides. The theranostic NPs were incubated with cells for different times, followed by washing with PBS. Then the red fluorescence signal of DOX and blue fluorescence signal of hoechst 3342 were observed a fluorescence microscope (Zeiss LSM 780).

**Figure S1.** TEM images of the (a) AuNR@IR780/DOX-RGD-PEG NPs, and (b) the hybrid NP treated with pH 6.5 acid solution (b) and pH 5.5 solution (c).
**Figure S2.** X-ray photoelectron spectroscopy (XPS) curves fit of Au$_{4f}$ spectra of the multifunctional AuNR@IR780/DOX-RGD-PEG NP before (red line) and after treated with acidic solution (pH 5.5) (black line). The signal of Au$_{4f}$ was not find from the AuNR@IR780/DOX-RGD-PEG NP, owing to the small AuNR was coated with a thick layer of IR780 and DOX.

**Figure S3.** Zeta potential of the hybrid NP (red bar) and after the NP treated by pH 6.5 solution (blue bar) and pH 5.5 solution (green bar).
Figure S4. PA signal of the hybrid NP solution irradiated by PA imaging laser for 10 times.

Figure S5. In vitro DOX release profiles of the theranostic NPs treated by GSH or H$_2$O$_2$. 
Figure S6. The DOX signal in the cell uptake of the thersnostic NPs after 0h, 1h, 3h, and 5h incubation by flow cytometry.

Figure S7. In vitro fluorescence images of $\alpha_v\beta_3$ negative MCF-7 cancer cells (a) and U87MG cells blocked by free RGD after incubated with the theranostic NP (b) at pH 6.5 cell culture solutions after 5 h post-incubation. (blue color: cell nuclei, red color: DOX). Scale bars: 50 $\mu$m.
**Figure S8.** The average number of AuNRs uptake by healthy cells (healthy MCF-10A) under different conditions.

**Figure S9.** Hydrodynamic diameter of the hybrid NP incubated in H$_2$O, PBS, cell culture medium, or cell culture medium plus 10% fetal bovine serum (FBS) before (a) and after 5 days (b). No obvious size variation was observed, indicating the excellent stability of the NPs in different physiological solutions.
Figure S10. Time-active curves of the biodistribution of AuNR@IR780/DOX-RGD-PEG NPs (a), AuNR@IR780/DOX NPs (b), AuNR@IR780/DOX-RGD NPs (c). Due to the PEG protection effect, the GNRs with PEG has a longer half-circulation time (t1/2=19 h) than that of AuNR@IR780/DOX NPs (t1/2=6.5 h) and AuNR@IR780/DOX-RGD NPs (t1/2=7.2 h).

Figure S11. PA imaging of the tumor region at 0, 6 and 24 h post-injection of the theranostic NPs, showing the distribution of the samples in the tumor tissue.
**Figure S12.** In vivo biodistribution of AuNR@IR780/DOX-RGD NPs (a) and AuNR@IR780/DOX-PEG NPs (b) in major organs and tissues of the tumor-bearing mice.

**Figure S13.** Relative MCF-7 and U87MG tumor volume of the tumor-bearing mice in different treatment conditions. MCF-7 tumor bearing mice was treated with the hybrid NP and laser irradiation. U87MG tumor-bearing mice was treated with free RGD first and then with the hybrid NP and laser irradiation.
Figure S14. Haematoxylin and eosin staining of different organ sections (heart, liver, spleen, lung and kidney) collected from different groups of U87-MG tumor-bearing mice after cancer therapy of 16 days.