Supplementary information

Experimental section

Chemicals and Materials

No additional purification with all commercially available chemicals and materials we utilized unless otherwise stated. The experiments were performed with deionized water (Millipore Milli-Q grade) with a resistivity of 18.2 M Ω . HAuCl₄ was bought from Sigma-Aldrich. Inc. Bovine serum albumin (BSA) with thiol groups was bought from from YareBio, Inc.

Synthesis of C-dots

C-dots were synthesized in consistent with our previous report.¹⁴ First, polythiophene phenylpropionic acid (PPA) was dissolved in alkaline solution. Then, the mixture was transferred to a stainless steel reactor and heated at 240 °C for 36 h. After cooling to room temperature, the resultant C-dot solution was filtered through Millipore 0.22 μ m filter paper and then dispersed in water.

Synthesis of C-dots@Au

First, 500 µg of C-dots were dispersed into 1 mL of pure water. Without any addition of reducers from outside environment, then various concentrations of HAuCl₄ solution (5, 10, 15, 20 mM) were added respectively. The mixture was vigorously stirred for 30 min at room temperature. After reaction, the obtained solution was centrifuged at 12000 rpm to obtain precipitate (C-dots@Au). To remove excess HAuCl₄ solution, C-dots@Au was washed thrice and re-dispersed into water.

Synthesis of BSA-coated C-dots@Au nanoflowers

5 mg of BSA with thiol groups was added into 2 mL of C-dots@Au nanoflowers aqueous solution (500 μ g mL⁻¹). The mixed aqueous solution was then stirred at room temperature for 12 h. After reaction, the obtained solution was centrifuged at 15000 rpm to obtain precipitate (C-dots@Au nanoflower). To remove excess BSA, C-dots@Au nanoflowers were washed thrice and re-dispersed into water.

Measurement of photothermal performance

Various concentrations (50, 100, 200, 300 µg mL⁻¹) of C-dots@Au nanoflowers were dispersed in water. Then, the C-dots@Au nanoflowers aqueous solution was placed in a quartz cuvette and irradiated by a 750 nm laser at a power density of 2 W cm⁻² for 10 min to measure the temperature elevation in the solution. The thermoelectric couple, which had an accuracy of 0.1 °C, was inserted into the solution in a position perpendicular to the light path. Temperature was recorded every 1 s by a digital thermometer with a thermoelectric couple.

Calculation of the photothermal conversion efficiency of C-dots@Au nanoflowers

The photothermal conversion efficiency (η) of the C-dots@Au nanoflowers was calculated from the energy balance formula (1):

$$\eta = \frac{hS\Delta T_{\max} - hs\Delta T_{H_2O}}{I(1 - 10^{-A750})} \quad (1)$$

$$\tau = \frac{m_D C_D}{hS} \tag{2}$$

where h is the heat transfer coefficient, S is the surface area of the container, ΔT_{max} (26.5 °C) is the maximum temperature change of the C-dots@Au nanoflowers from the equilibrium to the surroundings, and ΔT_{H2O} (7.1 °C) is the temperature elevation of deionized water. I (2 W cm⁻²) represents the laser power we utilized, A₇₅₀ (0.214) is the absorbance of the C-dots@Au nanoflowers at 750 nm. The value of hS (0.0137) is derived from Equation (2). τ is the time constant, m_D (1 g) and C_D (4.2 J g⁻¹) represent the mass and heat capacity respectively of the deionized water used as the solvent. According to the two equations, the τ value (306.6 s) was calculated from the slope by applying the linear time data from the cooling period versus the negative nature logarithm of driving force temperature, which was obtained from the thermoelectric couple. The η value of C-dots@Au nanoflowers was evaluated to be 22.5%.

FL imaging detection

HeLa cells were cultured at 37 °C in DMEM/F12 medium, which was supplemented with heat-inactivated fetal bovine serum (10%). Cells were seeded in 35 mm cell culture dishes and incubated with C-dots@Au nanoflowers at 50 µg mL⁻¹. After

stimulating for 4 h of incubation, the cells were washed twice with PBS to discard excess material. Images were captured with a Nikon C1si laser scanning confocal microscopy under excitation of 543 nm.

PA imaging detection

C-dots@Au nanoflowers with various concentrations (0, 50, 100, 200, and 300 µg mL⁻¹) were added into the agarose tube (37 °C) and under the same concentration of micelles as the control. Then they were scanned by a PA imaging instrument (Mode: iTheraMedical Co. MOST inVision 128; excitation wavelength ranged from 680-980 nm with 5 nm interval). Meanwhile, PA signals were recorded with a mean pixel intensity of the same area in the images.

MTT assay and photothermal therapy of cancer cells

HeLa cells were incubated with C-dots@Au nanoflowers (300 µg mL⁻¹) for 4 h and then were irradiated by a 750 nm laser (2 W cm⁻²) from 0 to 10 min. The cells were co-stained with calcein-AM and PI for 30 min, washed with PBS, and then imaged by a Nikon C1si laser scanning confocal microscopy. We incubated the Hela cells with various concentrations of C-dots@Au nanoflowers for the MTT assay to measure the cytotoxicity and the PTT efficacy of the C-dots@Au nanoflowers, as well as to investigate the cell viability. To estimate the cytotoxicity of the C-dots@Au nanoflowers, HeLa cells were seeded into 96-well plates and cultivated for 24 h with various concentrations (0, 50, 100, 200, and 300 µg mL⁻¹) of C-dots@Au nanoflowers at 37 °C with a 5% humidified atmosphere. For photothermal therapy of cancer cells, HeLa cells were cultured with C-dots@Au nanoflowers (0, 50, 100, 200, and 300 µg mL⁻¹) for 4 h at 37 °C and then irradiated with a 750 nm laser (2 W cm⁻²) for 10 min. After irradiation, the cells were incubated for another 24 h. The dark control group remained under the same conditions but was not treated with laser irradiation. The culture medium was discarded, and 80 µL of dimethylsulfoxide was added. Absorption was detected at 570 nm. The absorption detected for an untreated cell population under the same circumstance was used as the reference point to construct 100% cell viability.



Fig. S1 The elemental mapping of single C-dots@Au nanoflower on ultrathin carbon film by high-resolution TEM. Scale bare: 100 nm.



Fig. S2 High-resolution TEM image of the C-dots@Au nanoflower.

Zeta Potential Distribution



Fig. S3 Zeta potential distribution of C-dots@Au nanoflowers.



Fig. S4 TEM image of BSA-coated C-dots@Au nanoflowers.



Fig. S5 FTIR results of C-dots@Au nanoflowers and BSA-coated.



Fig. S6 DLS results of C-dots@Au nanoflowers and BSA-coated under a storage of two days.



Fig. S7 Confocal images of HeLa cells incubated with C-dots@Au nanoflowers. (a) Nuclei of HeLa cells stained with Dapi. Scale bar: 20 μ m. (b) Z-stack HeLa cell imaging.



Fig. S8 Confocal image of calcein-AM/PI co-staining HeLa cells without incubation of C-dots@Au nanoflowers by a 750 nm laser (2 W cm⁻²) irradiation for 10 min. Scale bare: 100 nm.



Fig. S9 Confocal image of calcein-AM/PI co-staining HeLa cells with incubation of C-dots@Au nanoflowers (300 μ g mL⁻¹) by a 750 nm laser (2 W cm⁻²) irradiation for 10 min. Scale bare: 100 nm.