Materials and Methods:

ESEM observation of MCF-7 spheroids and monolayer cells. To demonstrate the real shape of the spheroid, environmental scanning electron microscopy (ESEM) was used to obtain the outer morphology of the spheroid. After incubation for one week, the spheroid at the seeding concentration of 600 cells per well was taken out, washed with PBS, fixed overnight in 5% glutaraldehyde solution, and then washed with PBS. Afterwards, the spheroid was progressively dehydrated by gradient concentrations of ethanol from 70% to 100%, followed by critical point drying with isoamyl acetate. Finally, the spheroid without gold coating was observed with ESEM (FEI Quanta 200, Netherlands), which displayed the real morphology of the spheroid. For monolayer cells, cells were cultured on the cover slip for 24 h. Then, the medium was removed, and the cells was underwent the same treatment to that of the spheroid.

Flow cytometry. In order to quantitatively analyze the cell conditions in the tumor spheroid, the cells were stained by the Live/Dead assay kit (Molecular Probe, Carlsbad, CA). Briefly, about 15~30 spheroids were taken out, washed with PBS, and treated with 0.25% trypsin-EDTA at room temperature to produce single cell suspension. Afterward, the cell suspension was stained with calcein AM to a final concentration of 2 μM and ethidiumhomodimer (EthD-1) of 4 μM at room temperature for 20 min. Subsequently, the cells were centrifuged for 3 min at 2500 rpm to remove the dyes, re-suspended with the culture medium, and then analyzed by flow cytometer (BD FACSCalibur, USA). And the necrotic cells were observed as indicated by the red fluorescence from EthD-1 and the live cells by the green

fluorescence from calcein AM.

HE staining. After biodistribution study, tissue samples including tumor, heart, liver, spleen, lung, and kidney were collected and then fixed in 4% formalin at least one day. Then the samples were dehydrated with a series of ethanol, embedded in paraffin, and sectioned. Subsequently, sections were stained with hematoxylin and eosin (HE) according to the standard protocol.

ICP-MS. For the quantitative determination of Au content, samples including cell samples, blood sample, and tissue samples were digested in aqua regia composed of hydrochloric acid and nitric acid (3:1, v:v), diluted with 2% nitric acid and 1% hydrochloric acid, and then subjected to ICP-MS.

Bio-TEM observation of spheroids and tumors. In order to observe the inner cells of the spheroid, Bio-TEM observation of the spheroid was carried out. Briefly, the 7-day-cultured spheroid was taken out, washed with PBS, and then firstly fixed overnight at room temperature using 3% glutaraldehyde solution followed by secondary fixation with 1% osmium tetraoxide, and serial dehydration in gradient concentrations of ethanol. Subsequently, the spheroid was embedded in Epon resin and polymerized for 3 days at 60°C. Embedded samples were sectioned, and then stained with uranyl acetate. The samples were examined under an electron microscopy (JEM-1400, JEOL, Japan). For tumor tissues, small pieces of the tumor tissues were collected and underwent the same procedures mentioned above, unless the sections were not stained with uranyl acetate.

Supplementary Figure S1. Characterization of 15 nm Au@citrate nanoparticles. (A) size histograms of as-synthesized 15 nm Au nanopartices coated with citrate. (Scale bar is 100 nm) (B) Zeta-potential of the 15 nm Au@citrate nanoparticles. (C) Visible spectrum of the 15 nm Au@citrate at the wavelength between 400 nm and 800 nm, and the corresponding photograph of nanoparticles dispersed in pure water.

Supplementary Figure S2. (A) Representative TEM images of 50 nm and 100 nm Au@tiopronin NPs. (B) Photos of 50 nm and 100 nm Au@tiopronin NPs in water, 5% glucose, 0.9% saline, PBS and cell-culture medium (DMEM with 10% fetal bovine serum) containing phenol red. (C) The corresponding dynamic light scattering (DLS) and (D) visible spectrum of 50 nm and 100 nm Au@tiopronin NPs in water, 5% glucose, 0.9% saline, PBS and cell-culture medium (DMEM with 10% fetal bovine serum) containing phenol red.

Supplementary Figure S3. Cytotoxicity of MCF-7 monolayer cells after treated with 50 nm and 100 nm Au@tiopronin nanoparticles for 24 h, which is determined by CCK-8 assay. (Mean values ± standard deviation, n=4)

Supplementary Figure S4. Images of tissues including heart, lung, and kidney taken at 24 h after a single intravenous injection of 50 nm and 100 nm Au@tiopronin nanoparticles at a dose of 5 mg Au/kg after HE staining, and the corresponding tissue's images taken under dark field.

Supplementary Table S1. Average Au concentration in different organs (μg Au/g tissue) after 24 h administration of 50 nm and 100 nm Au@tiopronin nanoparticles at a dose of 5 mg Au/kg in tumor-bearing nude mice. Data represent mean ±S.D. (3 animals

per group)