Supplementary Information

Switching the subcellular organelle targeting of atomically precise gold nanoclusters by modifying the capping ligands

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Materials.

All reagents were obtained from commercial sources and used without further purification, including tetrachloroauric(III) acid ($HAuCl_4 \cdot 3H_2O$), GSH (γ-Glu-Cys-Gly), sodium cyanoborohydride ($NaBH_3CN$), methanol (MeOH), absolute ethanol, azobisisobutyronitrile (AIBN), 4-bromo-1-butene, thioacetic acid, ethyl acetate, triphenyl phosphine (PPh_3), sodium hydroxide (NaOH), hydrobromic acid (HBr), dichloromethane (DCM), toluene, petroleum ether, 3,3'-diaminobenzidine (DAB), 2',7'-dichlorofluorescein diacetate (DCFH-DA), Lysotracker green, Mitotracker green, 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), Dulbecco's Modified Eagle's Medium (DMEM), fetal calf serum, double resistance and trypsin-EDTA. Breast cancer (MCF-7) cells were obtained from Key Laboratory of Ecological Engineering and Biotechnology of Anhui Province, School of Life Science, Anhui University (Hefei, P. R. China). Pure water (Milli-Q, resistivity: 18.2 MΩ·cm) was used throughout the work. All glassware was thoroughly cleaned with aqua regia (HCl/HNO_3 =3:1 v/v), rinsed with plenty of pure water, and then dried in an oven prior to use.

Materials Characterization.

Transmission electron microscope (TEM) images were gained with a JEM-100SX (Japan Electronics, Japan) transmission electron microscope under an acceleration voltage of 200kV. Fourier transform infrared (FT-IR) spectra were obtained on a NEXUS-870 spectrometer (Thermo Fisher Scientific, USA). Ultraviolet visible (UV-Vis) absorption spectra were performed by a UV-3900 UV spectrophotometer (Hitachi, Japan). Fluorescence spectra were acquired with an F-4600 fluorescence spectrophotometer (Hitachi, Japan). Thermogravimetric (TG) analysis was obtained using a TGA-50 instrument. X-ray photoelectron spectroscopy (XPS) spectra were recorded using Thermo ESCALAB 250 spectrometer with Al Kα radiation (1486.6eV) as excitation source. Electrospray ionization mass spectrum (ESI-MS) was recorded using an Agilent 6530 Accurate-Mass Q-TOF mass spectrometer. The sample was directly infused into the chamber at 5μL/min. To prepare the ESI sample, clusters were dissolved in 1:1 water-MeOH solution (~0.1 mg/mL). ^1H NMR (D_2O as a solvent)

spectra were carried out on Bruker 400 Avance spectrometer. The OD values of MTT assay were measured by a RT-2100C spectro-photometric micro-plate reader (Rayto, P. R. China). Fluorescence images were recorded on a DMI3000B inverted fluorescence microscope (Leica, German). Confocal laser scanning microscope (CLSM) images were captured using an Olympus FluoView™ FV1000 confocal microscope with 60×oil immersion objective.

Experimental Section.

Synthesis of $Au_{18}SG_{14}$. In this experiment, $Au_{18}SG_{14}$ were prepared following the literature procedure with a little modification.[1] Briefly, 1.2 mL of MeOH, 1 mL of water and 300 mg of GSH were mixed in a 50 mL of round-bottom flask and stirred gently for 1min. Then, 750μL of $HAuCl_4 \cdot 3H_2O$ (0.2 $mg \cdot mL^{-1}$) was added. In the agitating process, the color of the solution changed from yellow to almost colorless. It was diluted to 30 mL by MeOH followed by the addition of 4.5 mL MeOH solution of $NaBH_3CN$ (220 mM). After 5h of vigorous stirring, the precipitate was collected and washed repeatedly with MeOH through centrifugal precipitation to remove the remaining precursors. Finally, the solution was freeze dried to obtain $Au_{18}SG_{14}$.

Synthesis of MTPB. MTPB was synthesized by the method shown in Scheme S1. Firstly, a mixture of 10.0 mg AIBN, 5.64 mL 4-bromo-1-butene (54 mM), and 7.9 mL thioacetic acid (108 mM) were dissolved in 100 mL of Shrek bottle filled with Ar at 40℃ under stirring for 80 min. The products were dissolved and washed by ethyl acetate and K_2CO_3 0.8 g/20 mL H_2O, successively. The generated organic phase was dried and filtered to afford yellow oil form things (thioacetic acid S-(4-bromo-butyl) ester, TABB) and purified by 200~300 silica gel column (2 x 20 cm, petroleum ether : ethyl acetate, 98 : 2). Secondly, 18.0 g PPh_3 and 24 mL toluene were mixed in a 250 mL three-necked bottle filled with Ar followed by the addition of 24 mL toluene solution of TABB (60.06 mM) under stirring for 2h at 110 ℃. The mixture was cooled down to room temperature, and then rest overnight. The generated yellow sticky solid was washed by ethyl acetate to remove unreacted PPh_3 and redissolved in 40

mL absolute ethanol. Then 170 mL of NaOH (0.1M) solution was added to adjust the pH to 9 followed by the addition of HBr to adjust the pH to 4. The final product (MTPB) was filtered, extracted by DCM and washed by petroleum ether to afford pale yellow solid. Yield: 72.7%.

Synthesis of $Au_{18}SG_{12}MTPB_2$. 10 mg $Au_{18}SG_{14}$ dissolved in 4.5 mL pure water was added in a 25 mL round bottomed flask under a slow agitation followed by the addition of 0.5 mL MeOH solution of MTPB (2 mM). After 2h of slow agitation, the product was collected through centrifugal precipitation and the solution was freeze dried to obtain $Au_{18}SG_{12}MTPB_2$.

Measurements of singlet oxygen ($^1O_2$) generation under laser irradiation at 638 nm. $^1O_2$ generation of the nanoclusters was measured by using DAB as an indicator. Briefly, the nanoclusters in PBS (400μg/mL) containing 500 μM DAB was irradiated by a 638 nm laser with a power intensity of 1W/cm^2. The adsorption spectra were recorded after different periods of light irradiation.

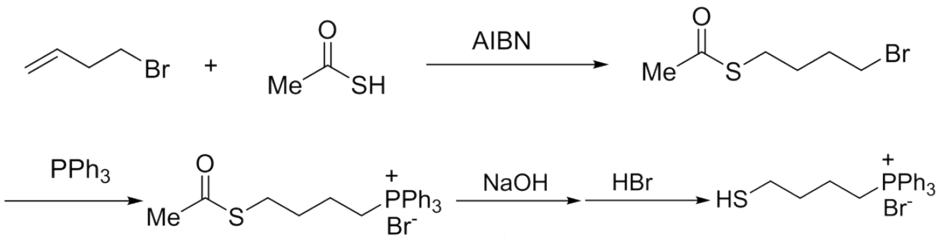
In addition, the intracellular $^1O_2$ production of nanoclusters in MCF-7 cells was examined by using the DCFH-DA as a $^1O_2$ fluorescence indicator. In briefly, MCF-7 cells were seeded into an 6-well glass bottom plate with a density of 2×10^4 cells per well. After 24 h of incubation, the medium was replaced with fresh medium containing $Au_{18}SG_{14}$ or $Au_{18}SG_{12}MTPB_2$ and further incubated for 6 h. The cells were washed with serum-free DMEM and incubated with DCFH-DA (10 μM) for 20 min. After washing twice with PBS, cells were irradiated with a 638 nm laser (1W/cm^2, 10 min). Then the cells were immediately examined under an inverted fluorescence microscope.

In vitro cell cytotoxicity and photodynamic-induced cell death. The MTT assay was used to investigate the *in vitro* toxicity of nanoclusters. MCF-7 (5000 per well) in 100 μL of culture medium were placed in the wells of 96-well plates and grown overnight. Then, the medium was removed and 200 μL of fresh culture medium containing nanoclusters at different concentrations (0, 50, 100, 200, 300, 400 and 500 μg·mL^{-1}) was introduced to the cells for 24h, respectively, at 37°C under 5% CO_2/95% air. The remaining processes are the same as the general MTT assay.[2] All of the tests were

independently performed six times.

MTT was also used to study the photodynamic toxicity of nanoclusters against MCF-7 cells. In a typical experiment, MCF-7 cells were first seeded into a 96-well plate at a density of 1×10^4 cells per well at 37 °C in a 5% CO_2 atmosphere for 24 h. The cells then were washed three times with PBS to remove dead cells, followed by incubation with same concentrations (100μg·mL^{-1}) of $Au_{18}SG_{14}$ and $Au_{18}SG_{12}MTPB_2$ dispersed in DMEM medium at 37 °C for 2 h. Next, the cells were washed three times with PBS to remove free nanoclusters. Then, fresh culture medium was added, and the cells were exposed to 638 nm laser (1 W/cm^2) for various length of time (5 min, 10min, 15min, 20min, 25min), respectively. After laser irradiation, the cells were further incubated with fresh DMEM culture medium at 37 °C for 24 h. The cell viability then was also determined by general MTT.[2]

CLSM bioimaging and colocalization. MCF-7 cells were seeded onto a Φ=35 mm confocal laser dish at a density of 5×10^4 cells per dish and grown at 37°C for 12 h. The medium was then replaced with 1mL of fresh medium containing nanoclusters (100 μg·mL^{-1}) to further incubating at 37°C for 1 h, respectively. After being washed two times with PBS, Lysotracker green (60 nM) or Mitotracker green (100 nM) was used for staining for 20 min. Finally, the cells were washed twice with PBS, and differential interference contrast (DIC) and fluorescent images were taken with CLSM.



Scheme S1 Synthetic routes for MTPB.

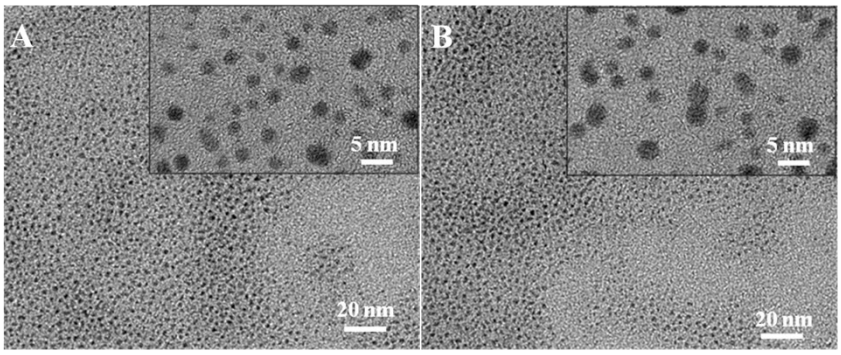


Fig. S1 TEM images of (A) $Au_{18}SG_{14}$ and (B) $Au_{18}SG_{12}MTPB_2$.

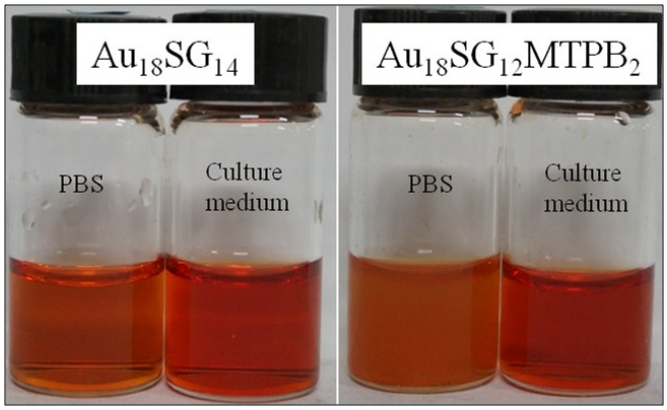


Fig. S2 Typical pictures of $Au_{18}SG_{14}$ and $Au_{18}SG_{12}MTPB_2$ solution in PBS and culture medium, respectively.

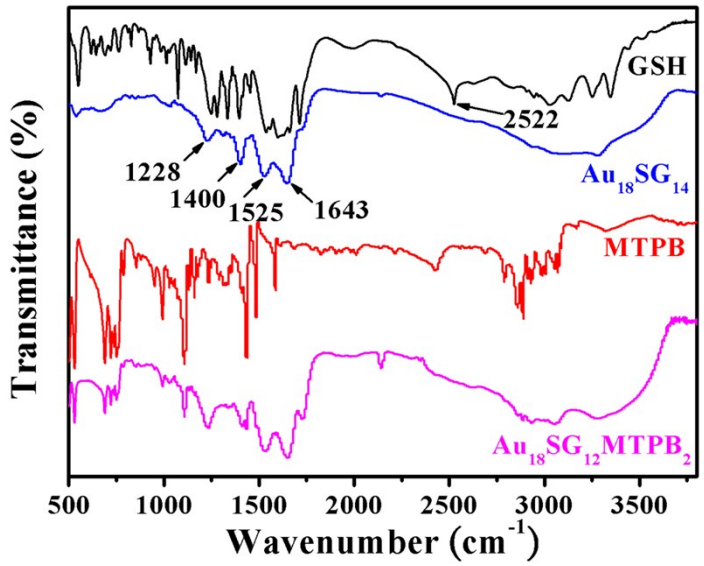
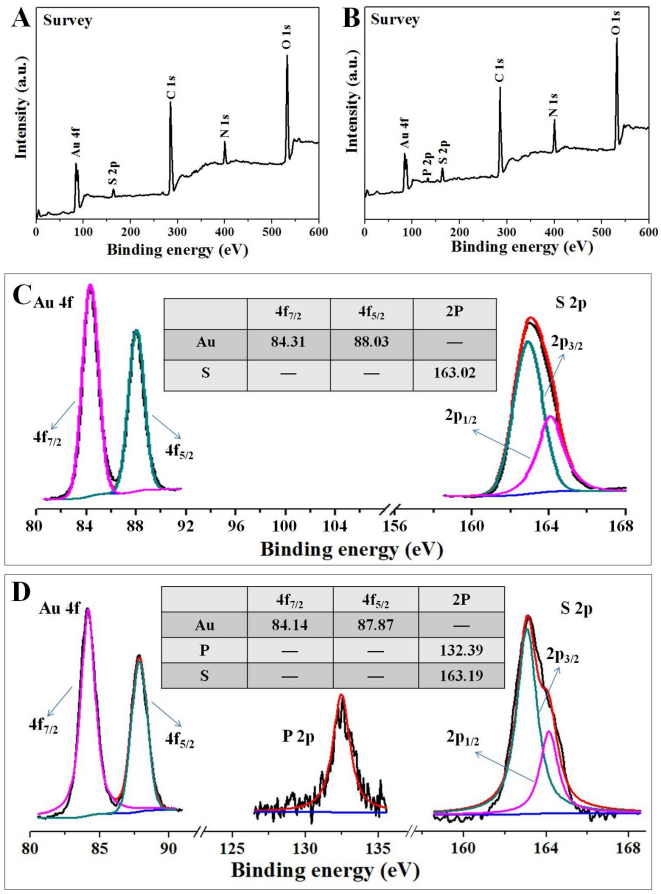


Fig. S3 FTIR spectra of GSH, MTPB, $Au_{18}SG_{14}$ and $Au_{18}SG_{12}MTPB_2$.



	$4f_{7/2}$	$4f_{5/2}$	2P
Au	84.31	88.03	—
S	—	—	163.02

	$4f_{7/2}$	$4f_{5/2}$	2P
Au	84.14	87.87	—
P	—	—	132.39
S	—	—	163.19

Fig. S4 XPS survey spectra of the as synthesized (A) $Au_{18}SG_{14}$ and (B) $Au_{18}SG_{12}MTPB_2$. XPS spectra for (C) $Au_{18}SG_{14}$ of Au 4f, S 2p core levels and (D) $Au_{18}SG_{12}MTPB_2$ of Au 4f, P 2p, S 2p core levels. Peaks are fitted after background subtraction. Inset in Fig. S3-C: table presenting the binding energy values of gold and sulfur. Inset in Fig. S3-D: table presenting the binding energy values of gold, phosphorus and sulfur.

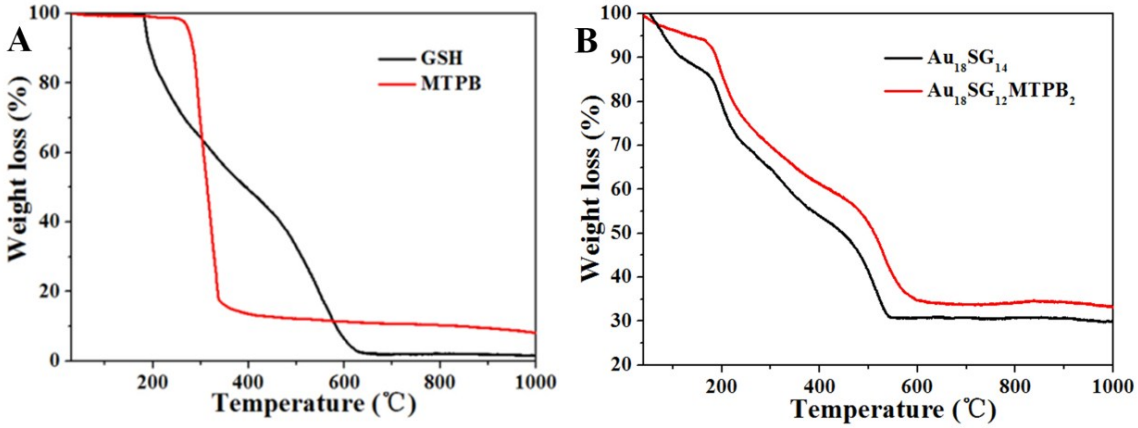


Fig. S5 TG curves of GSH, MTPB, $Au_{18}SG_{14}$ and $Au_{18}SG_{12}MTPB_2$.

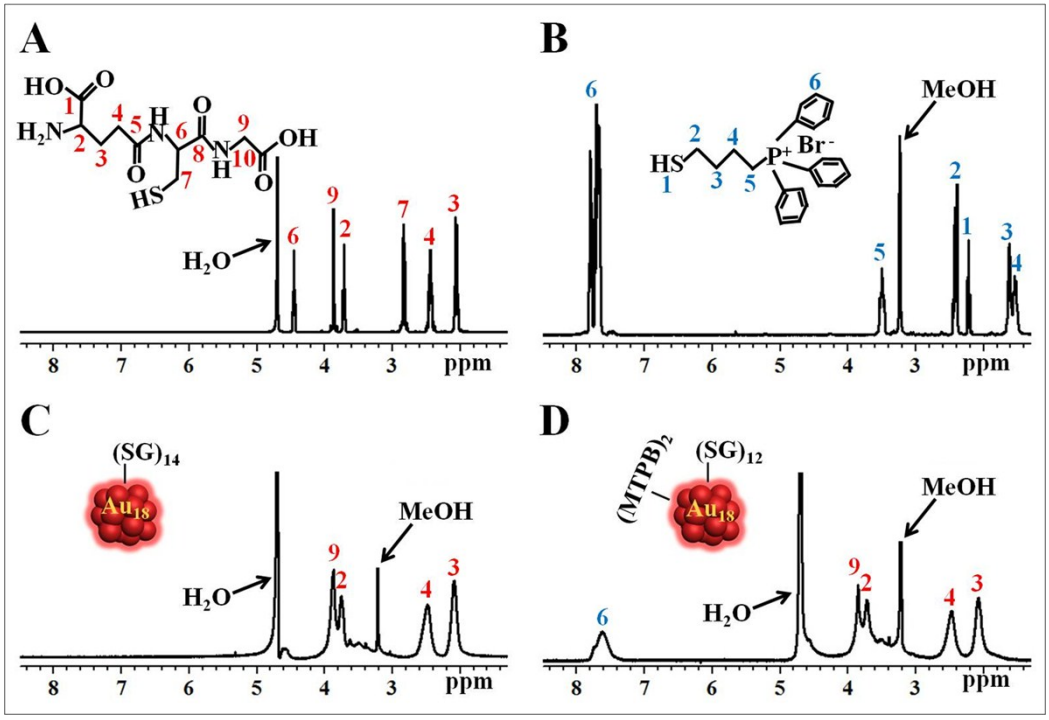


Fig. S6 ^{1}H NMR spectra of (A) GSH, (B) MTPB, (C) $Au_{18}SG_{14}$ and (D) $Au_{18}SG_{12}MTPB_2$.

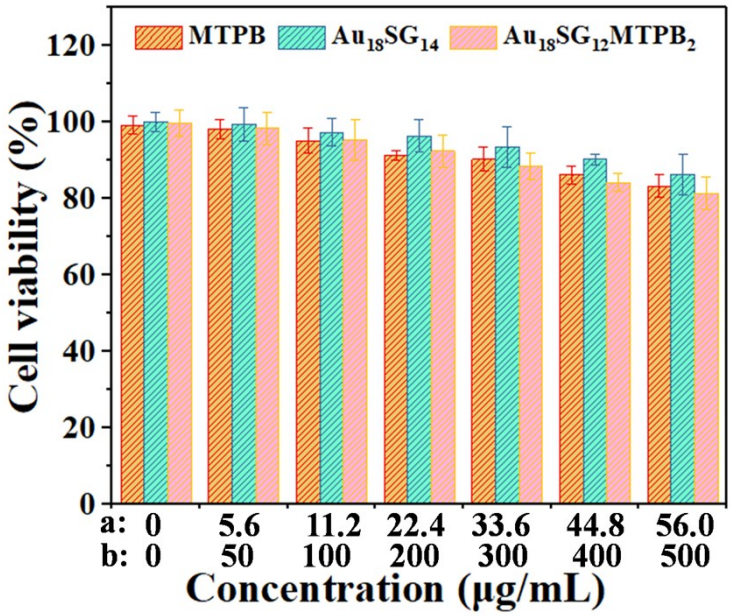


Fig. S7 *In vitro* cytotoxicity studies of MTPB, $Au_{18}SG_{14}$ and $Au_{18}SG_{12}MTPB_2$ performed on MCF-7 cells at different concentrations after 24 h incubation. Legend: a refers to the concentration of MTPB and b refers to the concentration of nanoclusters.

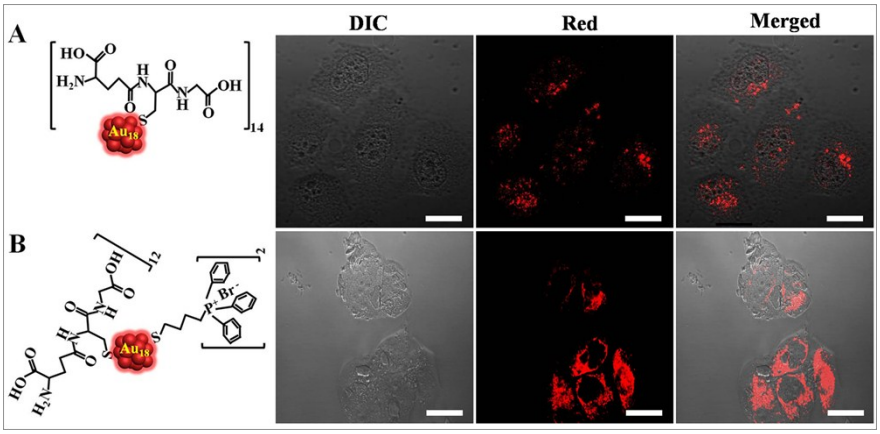


Fig. S8 CLSM images of live MCF-7 cells treated with 100 μg/mL (A) $Au_{18}SG_{14}$ and (B) $Au_{18}SG_{12}MTPB_2$ for 1 h at 37 °C, respectively. Images were captured in DIC mode and red channel. All scale bars are 20μm.

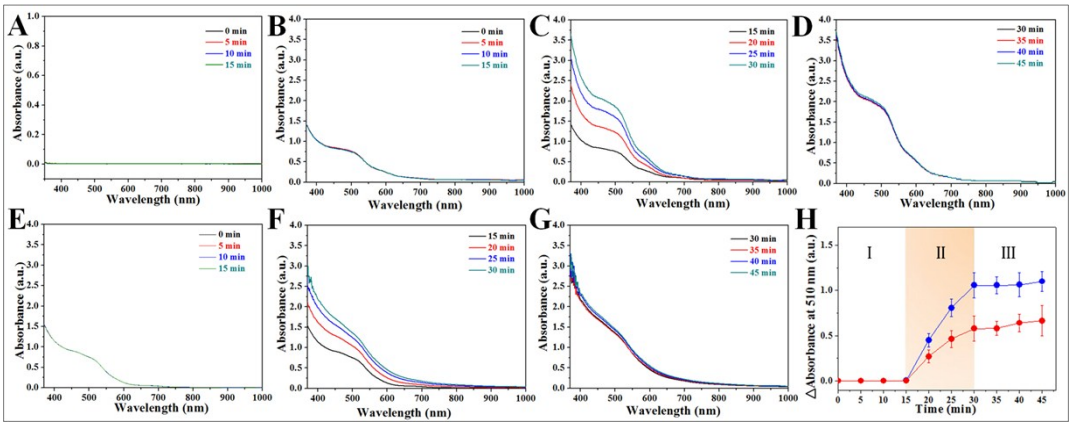


Fig. S9 (A) Absorption spectrum of DAB in PBS in the absence of nanoclusters under a 638nm red laser irradiation for 15 min. Absorption spectra of a DAB-containing solution of (B-D) $Au_{18}SG_{14}$ or (E-G) $Au_{18}SG_{12}MTPB_2$ in PBS: (B, E) 0-15 min under darkness, (C, F) 15-30 min under a 638nm red laser irradiation (1.0 $W \cdot cm^{-2}$) and (D, G) 30-45min under a 638nm red laser irradiation (1.0 $W \cdot cm^{-2}$) in the presence of histidine (20 mM). (H) Changes in absorbance at 510 nm of a DAB-containing solution of $Au_{18}SG_{14}$ (blue) and $Au_{18}SG_{12}MTPB_2$ (red) in PBS. Region I, darkness; region II, laser irradiation at 638 nm; and region III, addition of histidine scavenger.

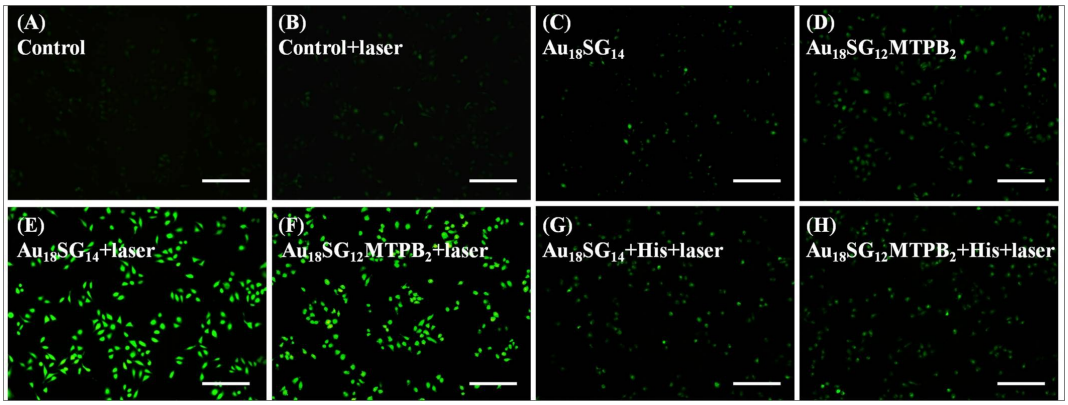


Fig. S10 Fluorescence microscopy images of MCF-7 cells that received different treatments as indicated. Green color represents $^1O_2$ indicator DCFH-DA (scale bar=200 μm). [Legend: (A) none treated cells as a control; (B) cells treated only with 638 nm laser irradiation; cells treated with (C) $Au_{18}SG_{14}$ or (D) $Au_{18}SG_{12}MTPB_2$ without laser irradiation; cells treated with (E) $Au_{18}SG_{14}$ or (F) $Au_{18}SG_{12}MTPB_2$ with 638 nm laser irradiation; cells treated with (G) histidine/$Au_{18}SG_{14}$ and (H) histidine/$Au_{18}SG_{12}MTPB_2$ with 638 nm laser irradiation.] The 638 nm laser power intensity is 1 W/cm^2. The untreated control cells showed almost no DCFH-DA fluorescence, indicating that few $^1O_2$ was present inside the cancer cells. Meanwhile, either the laser irradiation alone at 638 nm or the presence of nanoclusters alone without laser irradiation can only induce a negligible amount of $^1O_2$ inside the tumor cells, which was indicated by almost the same weakness of green fluorescence intensity in these groups, in comparison to the control group. However, when the nanocluster-treated cells were irradiated by 638 nm laser, we could clearly see strong green fluorescence in both nanocluster-treated cells. Furthermore, there was no more $^1O_2$ generation in the cells treated with nanoclusters in the presence of histidine under the 638 nm irradiation, implying that the effective scavenging effect of histidine on $^1O_2$ species. These results proved that the $^1O_2$ could be selectively generated in the Au_{18} nanocluster-treated cells upon 638 nm irradiation.

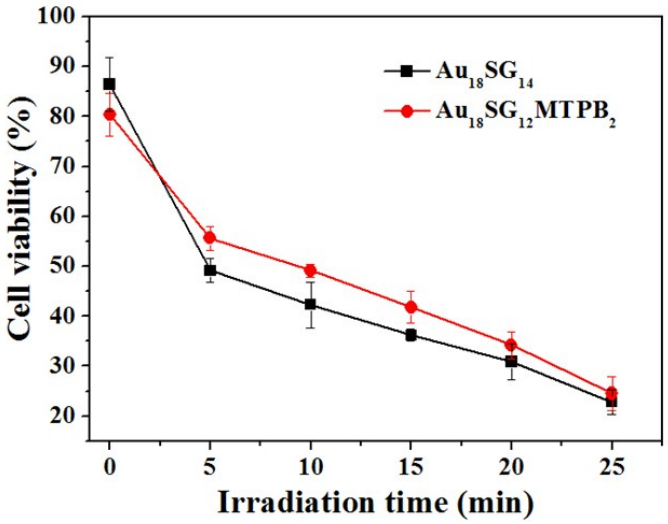


Fig. S11 In vitro photodynamic cytotoxicity studies of $Au_{18}SG_{14}$ and $Au_{18}SG_{12}MTPB_2$ at the concentration of 500μg/mL performed on MCF-7 cells under irradiation with 638 nm light for various time (0, 5, 10, 15, 20 and 25 min). Error bars are based on triplet measurements.

Notes and references

1 A. Ghosh, T. Udayabhaskararao and T. Pradeep, *J Phys. Chem. Lett.*, 2012, **3**, 1997-2002.

2 Y. Wang, B. Zhang, L. Zhu, Y. Li, F. Huang, S. Li, Y. Shen and A. Xie, *Acs Appl. Mater. Inter.,* 2014, **6**, 15000-15006.