Supporting Information

# The Gold Nanorod Functioned with the Glutathione Response Near-Infrared Fluorescent Probe as a Promising Nanoplatform for Fluorescence Imaging Guided Precision Therapy

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#### **1.1 Instruments**

Mice fluorescence imaging was performed on Perkinelmer IVIS Lumina XRMS Series III In Vivo Imaging System. Mice IR thermal imaging was performed by Infrared Thermal Camera (TESTO 865). Fluorescence spectra were obtained by a HORIBA Scientific Fluoromax-4 spectro fluorometer with a Xenon lamp and 1.0-cm quartz cells. Absorption spectra were measured on Thermo Scientific NanoDrop 2000/2000C spectrophotometer. Mass spectra were taken on LCQ Fleet LC-MS System (Thermo Fisher Scientific). All pH measurements were performed with a basic pH-Meter PH-3C digital pH-meter (Lei Ci Device Works, Shanghai) with a combined glass-calomel electrode. <sup>1</sup>H NMR, <sup>13</sup>C NMR spectra were recorded on a Bruker AVANCE IIITM 500 spectrometer. MTT Assay was carried out by a microplate reader (Tecan, Austria). The fluorescence images of cells were taken using a confocal laser scanning microscope (Japan Olympus Co., Ltd) with an objective lens (×60). Flow cytometry and intracellular fluorescence detection was carried out on flow cytometry (Aria, BD) with excitation at 633 nm and emission at 750-810 nm. The mean particle size was determined by DLS Zetasizer Nano ZS90 (Malvern Instruments Ltd, UK) and by TEM (JEOL, model JEM-1230, Japan).

## **1.2 Materials**

Water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore, Bedford, MA, USA). The stock solutions of AuNRs, CyPT-AuNRs were solute in ultrapure water and maintained in refrigerator at 4 °C. All solvents were purchased from Sinopharm Chemical Reagent Co., Ltd. Other chemicals were purchased from Sigma-Aldrich unless otherwise stated and straightforward used without further purification, unless otherwise stated. The purity of Cy-PT was separated on a Shimadzu LC-20AT HPLC system equipped with fluorescence and UV-vis absorption detectors. HEPES was obtained from Aladdin. All reactions were performed under argon protection and dark, monitored by TLC (Hailang, Yantai). Flash chromatography was carried out using silica gel (300-400 mesh). Human lung cancer cell lines (A549 cells) and human normal lung fibroblast cells lines (MRC-5) were obtained from the Committee on Type Culture Collection of the Chinese Academy of Sciences. The antibody of caspase 3, caspase 9, Hsp70, cytochrome *c* and  $\beta$ -Actin were obtained from Cell Signaling Technology (Beverly, MA. USA).

## 1.3 Spectroscopic Methods.

UV-visible spectra were obtained with 1.0-cm glass cells. CyPT-AuNRs (ultrapure water, 60 µg mL<sup>-1</sup>) and AuNRs (ultrapure water, 60 µg mL<sup>-1</sup>) were added to a 10.0-mL color comparison tube, and with HEPES buffer (10 mM, pH 7.4), then different concentrations of GSH was added. The mixture was incubated at 37 °C for 5 min before measurement. Fluorescence spectra were obtained with a Xenon lamp and 1.0-cm quartz cells. CyPT-AuNRs (ultrapure water, 60 µg mL<sup>-1</sup>) and AuNRs (ultrapure water, 60 µg mL<sup>-1</sup>) were added to a 5.0-mL color comparison tube. After with HEPES buffer (10 mM, pH 7.4), different concentrations of GSH were added. The mixture was incubated at 37 °C for 2 min before measurement. Then the fluorescence emission spectra of CyPT-AuNRs and AuNRs were integrated from 830 to 900 nm with excitation at 810 nm.

## 1.4 Singlet Oxygen Generation and Quantum yield.

In all the experiments, the irradiation intensity was fixed at 1.5 W/cm<sup>2</sup>. Singlet oxygen generation and singlet oxygen quantum yield ( $\Phi_{\Delta}$ ) were measured using Singlet oxygen sensor green (SOSG, Invitrogen co., USA) as a singlet oxygen probe and CyPT-AuNRs in DMF as a reference ( $\Phi_{\Delta(CyPT-AuNRs)}$ ) = 0.56).<sup>[1]</sup> Briefly, 60 µg/mL of CyPT-AuNRs were dissolved in ultrapure water and added with SOSG (1 µM) in water containing 2% methanol,

respectively. Then, the mixture solutions were irradiated for 5 min (808 nm, 1.5 W/cm<sup>2</sup>). The fluorescence intensity of the irradiated solution was promptly determined by NIR fluorescence spectrometer. The emission from 500-600 nm was afforded by exciting with a light resource of 494 nm wavelength and quantified for the singlet oxygen generation. CyPT-AuNRs in water at the concentration of 60 µg/mL were mixed with 30.0 µM DPBF, followed by 300 s irradiation (808 nm laser). Then, the absorbance of DPBF at 415 nm was monitored at various times. The value of  $\Phi_{\Delta}$  was calculated according to the equation of  $\Phi_{\Delta(cyPT-AuNRs)}$  W I<sub>CyPT-AuNRs</sub> /(W<sub>CyPT-AuNRs</sub> I<sub>sample</sub>),<sup>[2]</sup> where W<sub>sample</sub> and W<sub>CyPT-AuNRs</sub> are the DPBF photobleaching rates in the presence of sample and CyPT-AuNRs, respectively, and I<sub>sample</sub> and I<sub>CyPT-AuNRs</sub> are the rates of light absorption by sample and CyPT-AuNRs, respectively.

# 1.5 Photothermal Effect and Photothermal Conversion Efficiency.

The solutions of CyPT-AuNRs at the concentrations of 60  $\mu$ g/mL in 0.5 mL glass vials were irradiated by 808 nm laser for 5 min. Meanwhile, the temperatures of solutions were recorded using a thermometer and infrared thermal camera at an interval of 30 s. To assess the photothermal conversion efficiency, the solutions of CyPT-AuNRs, free CyPT, free AuNRs and CyPT-AuNRs + GSH in quartz cuvettes were irradiated at 808 nm. When the temperature reached a plateau, the irradiation was removed for cooling down to room temperature. The temperature of the solutions was recorded at an interval of 30 s, and then their photothermal conversion efficiencies were calculated.

#### 1.6 Photostability.

To evaluate the ability to maintain the temperature elevations under irradiation, 60 µg/mL CyPT-AuNRs were respectively illuminated at 808 nm for 5 min (LASER ON), and then the irradiation was removed for cooling the samples to room temperature (LASER OFF). Subsequently, another four cycles of this LASER ON/OFF cycles were carried out. During this process, the temperature was recorded at an interval of 30 s.

# 1.7 Cell Lines and Culture.

Human lung cancer cell lines (A549 cells) and human normal lung fibroblast cells lines (MRC-5) used in this study were purchased from Committee on Type Culture Collection of the Chinese Academy of Sciences. The cells were cultured in recommended media with 10% FBS and 1% penicillin/streptomycin, incubated in a humidified incubator (Thermo Scientific 3111, USA) at 37 °C with 5% CO<sub>2</sub>.

# 1.8 Determination of GSH Levels In Tumor Cells And Normal Cells.

Samples of  $1 \times 10^5$  cells (A549 cells and MRC-5 cells) were centrifuged and harvested, and then the pellets were washed with 200 µL of sterilized ice-cold  $1 \times$  PBS (pH 7.4) two times. The cell pellets were resuspended with 160 µL of sterilized ice-cold deionized water, followed by the addition of 160 µL of sterilized ice-cold 20 mM HCl and 80 µL of sterilized ice-cold 5% (wt/vol) 5-sulfosalicylic acid. The suspensions were transferred to 2 mL snap-cap tubes with 0.5 g acid-washed glass beads, and the cells were ruptured with a Mini-Bead beater-16 (BioSpec) for 1 min on and 2 min pause on ice, and repeated once. Then, the tubes were centrifuged at 13,000 g, at 4°C, for 15 min, and the supernatants were collected in new centrifuge tubes. Samples were then processed and the intracellular GSH concentrations were measured as described in the protocol of the Total Glutathione Assay Kit (Beyotime Biotechnology, China) by a Cytation3 Multi-mode Reader from BioTek.

### 1.9 Cell Staining Procedures and Colocalization-imaging Experiments.

The cells were plated on 6-well plates and allowed to adhere at 37 °C, 5% CO<sub>2</sub>, 24 h before imaging. The culture medium was then removed, and the cells were washed once with 1 mL of Dulbecco's Modified Eagle Medium (DMEM). A549 cells were placed in 1 mL of DMEM and loaded with 60 µg/mL CyPT-AuNRs for 3 h, washing the cells three times with DMEM to remove the excess CyPT-AuNRs. Finally, 1 µg/mL mitochondrial tracker (MitoTracker Green FM) (Thermo Fisher Scientific Inc.) was added and the cells were incubated for another 10 min at 37°C. Finally, the cells were rinsed with DMEM three times and mounted on the microscope. Fluorescent images were acquired on an Olympus Fluo View FV1000 confocal laser-scanning microscope (Japan) with an objective lens (×60). The spectrally separated images acquired from the three dyes were estimated using Image-Pro Plus software.

# 1.10 Flow cytometry.

FCM assay was carried out for the detection of the intracellular CyPT-AuNRs. A549 cells and MRC-5 cells were cultured at  $2.0 \times 10^5$  cells/well in 6-well plates, and treated with 60 µg/mL CyPT-AuNRs for 3 h at 37 °C. After harvest, cells were washed, and resuspended in PBS and analyzed by flow cytometry. And then the cells were further exposed to 808 nm laser light irradiation (1.5 W/cm<sup>2</sup>) for 5 min after incubated with singlet oxygen sensor green (SOSG, Invitrogen co., USA) for 20 min at 37 °C. After harvest, cells were washed, and resuspended in PBS and analyzed by flow cytometry.

# 1.11 Cytotoxicity of CyPT-AuNRs.

The cytotoxicity of CyPT-AuNRs was assessed by the MTT assay. A549 cells were seeded into 96-well cell culture plate at a final density of  $8 \times 10^3$  cells/well. And then different concentrations of CyPT-AuNRs (20µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, 100 µg/mL) were added to the wells. The cells were then incubated for 24 h at 37 °C under 5% CO<sub>2</sub>. Subsequently, MTT was added to each well (final concentration 5 mg/mL) for an additional 4 h at 37 °C under 5% CO<sub>2</sub>, then formazan crystals which were dissolved in 150 µL DMSO formed. The amount of MTT formazan was qualified by the absorbance (OD) at 570 nm using a microplate reader (Tecan, Austria). Calculation of IC<sub>50</sub> values were done according to Huber and Koella. The results are the mean standard deviation of six separate measurements.

# 1.12 In Vitro Synergetic PDT, PTT Effect of CyPT-AuNRs.

To evaluate ynergetic PDT and PTT effects of CyPT-AuNRs, A549 cells were used. Firstly,  $3 \times 10^3$  cells were seeded in 96-well plates and cultured overnight and then incubated with different concentrations (20µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, 100 µg/mL) of CyPT-AuNRs for 24 h. Cells were exposed to 808 nm laser irradiation (1.5 W/cm<sup>2</sup>) for 5 min. In the control group, cells were incubated with different concentrations of CyPT-AuNRs straightforwardly for 48 h without laser irradiation. The dose-dependent synergetic anticancer effect of CyPT-AuNRs were further intuitionally verified on A549 cells by using Calcein AM and PI co-staining. The green from Calcine AM and the red from PI showed the live and dead cells respectively, confirmed the synergistic effects of PDT and PTT. After 24 h incubation, the relative cell viabilities were determined by the MTT assay. In order to investigate the effect of PDT or PTT,<sup>[3]</sup> the cells were maintained below 4 °C by ice treatment to scavenge photothermal conversion or treated with 10 mM N-acetylcysteine (NAC, ROS scavenger) to scavenge ROS, during laser irradiation. We used the dark group (no laser irradiation) to investigate the single effect of chemotherapy by MTT assay. And Calcein AM and propidium iodide (PI) co-staining were carried out on A549 cells to further confirm the synergistic PDT/PTT effects of CyPT-AuNRs. Briefly, 5 × 10<sup>4</sup> A549 cells

per well were seeded in 6-well plates and cultured overnight. Then cells were incubated with 60  $\mu$ g/mL CyPT-AuNRs for 3 h. Afterward, the cells of experimental groups (single group of PDT, PTT; synergistic PDT/PTT group) were irradiated by an NIR (808 nm) laser with energy density of 1.5 W/cm<sup>2</sup> for 5 min. Incubation 3 h later, cells were stained with 2  $\mu$ M Calcein AM and with 2  $\mu$ M PI for visualization of live cells or visualization of dead/late apoptotic cells respectively. Cellular apoptosis and necrosis were evaluated by Annexin V-FITC Apoptosis Detection Kit and observed by flow cytometry. Lastly, the fluorescent images of cells in all groups were acquired with a biological inverted microscope after being rinsed with PBS.

# 1.13 Single Oxygen Detection in Lving Cells.

Singlet oxygen sensor green (SOSG, Invitrogen co., USA) was employed to evaluate the singlet oxygen generation of CyPT-AuNRs in living cells. A549 cells were treated with 60  $\mu$ g/mL CyPT-AuNRs and then added with SOSG (1  $\mu$ M) in water containing 2% methanol. The cells were irradiated for 5 min (808 nm, 1.5 W/cm<sup>2</sup>). The fluorescence imaging of the A549 cells were promptly determined by laser scanning confocal microscope. The emission from 500-600 nm was afforded by exciting with a light resource of 494 nm wavelength and quantified for the singlet oxygen generation.

# 1.14 In Vivo Imaging of BALB/c mice.

Six to eight-week-old BALB/c mice were obtained from Binzhou Medical University. Mice were grouphoused on a 12:12 light-dark cycle at 22 °C with free access to food and water. Mice were anesthetized prior to injection and during imaging via inhalation of isoflurane. BALB/c mice (20-25 g) were given intravenous injection of CyPT-AuNRs (60 µg/mL). Then the mice were imaged by using Perkinelmer IVIS Lumina XRMS Series III In Vivo Imaging System, with an excitation filter of 810 nm and an emission of 830-900 nm. The results were the mean standard deviation of five separate measurements. Additionally, we merged the fluorescence image with the corresponding bright field image to clearly display the reaction site of the mice. All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals, and protocols were approved by the Institutional Animal Care and Use Committee in Binzhou Medical University, Yantai, China.

## 1.15 Establish A549 Tumor Xenografts Models

The culture flasks were covered with human lung cancer cell lines (A549 cells), and then we shifted the cells and cell culture medium to centrifuge tubes in a super clean bench. The cells were centrifuged at 1000 r/min for 10 min and discarded the supernatant. We adjusted the cell concentration by Hanks solution to cause A549 tumor model. Athymic nude mice (aged 5-6 weeks, weighted 20-25 g) were purchased from Binzhou Medical University. All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals, and protocols were approved by the Institutional Animal Care and Use Committee in Binzhou Medical University, Yantai, China. For A549 tumor xenografts,  $5 \times 10^6$  A549 cancer cells suspended in 200 µL PBS were subcutaneously implanted into the right flank of each athymic nude mouse. All animals were maintained under aseptic conditions and were housed in a group of five in standard cages with free access to food and water and a 12 h light/dark cycle. The tumors were allowed to grow for about 8-10 days until the tumor diameter reached 8-10 mm.

#### 1.16 In vivo anticancer effect of CyPT-AuNRs under light irradiation.

To determine the in vivo tumor inhibitory effect of CyPT-AuNRs, A549 tumor xenograft models were used.

After tumor xenografts were established, they were randomly divided into four groups in each xenograft model: PBS non-irradiation group, PBS irradiation group, CyPT-AuNRs non-irradiation group and CyPT-AuNRs irradiation group. Animals in the CyPT-AuNRs non-irradiation group and CyPT-AuNRs irradiation group were intravenous injection CyPT-AuNRs (60  $\mu$ g/mL, 200  $\mu$ L in saline), every two days and repeated for 5 times. Animals in the PBS non-irradiation group and PBS irradiation group were given the same volume of blank PBS each time. Post-injection, tumors in the irradiation groups were exposed to an 808 nm laser at the power density of 1.5 W/cm<sup>2</sup> for 5 min. Tumor volumes and body weights were recorded during the treatments. Tumor volumes were calculated as length × (width)<sup>2</sup>/2. 2-3 days post fifth drug injection, animals were sacrificed, and tumors were dissected and weighted. Meanwhile, tumors in each group were collected and fixed in 4% formaldehyde, made into paraffin sections for H&E staining. The major organs from each group were also collected for H&E staining to determine the toxicside effect of this treatment.

# 1.17 In Vivo Tumor NIR Fluorescent Imaging and Thermal Imaging

The animals with A549 tumor xenograft models were used for tumor-targeted NIR imaging after intravenously injected 60 µg/mL CyPT-AuNRs with the Perkinelmer IVIS Lumina XRMS Series III In Vivo Imaging System. NIR imaging of whole animals in vivo was performed at different times (0.5 h, 6 h, 24 h, 48 h, 72 h) post drug injection. After sacrifice of the mice at 0.5 h, 6 h, 24 h, 48 h, 72 h, major organs and tumors were dissected for ex vivo NIR fluorescent imaging, and for studying their tissue distribution. For in vivo assessment of PTT property, mice with A549 tumor xenografts were intravenously injected CyPT-AuNRs (60 µg/mL). After injection, the real-time temperature change of mice was imaged by the infrared thermal camera (Testo 865, Germany) when the whole tumor tissue was exposed to the continuous NIR laser beam (808 nm, 1.5 W/cm<sup>2</sup>,0 - 5 min). Prior to NIR fluorescence or thermal imaging, mice were anesthetized by inhalation of isoflurane.

# 1.18 Western Blot.

 $1 \times 10^6$  A549 cells were seeded in 6-well plate and incubated overnight. They were treated with 60 µg/mL CyPT-AuNRs for 24 h incubation. Then, cells were irradiated with NIR laser light (808 nm, 1.5 W/cm<sup>2</sup>) for 0 min, 1 min, 3 min and 5 min respectively. For comparison, cells without incubation of CyPT-AuNRs was used for negative control, while cells with incubation of CyPT-AuNRs in the dark group (without laser irradiation) as the positive control. After 5 min laser irradiation, all cells were washed with PBS, protein extracts were prepared by suspending the cells in 200 µL RIPA lysis buffer containing 1% PMSF (Solarbio, China) and 20% PhosSTOP (Roche, Germany). Then the extracts were quantified with BCA protein assay kit (Biogot, China). After denatured, the equal amounts of protein were electrophoresed on 10% SDS-polyacrylamide gels (Bio-Rad, USA) and transferred to PVDF membranes. The membrane was incubated with 5% BSA (Sigma-Aldrich, USA) and incubated with primary antibodies overnight at 4 °C with gentle shake. A horseradish peroxidase (HRP)-conjugated secondary antibody (Cell Signaling Technology, USA) was used to mirror the quantity of proteins and signals were detected with an enhanced chemiluminescence (ECL) detection system. The results were analyzed by Image J to acquire the grey value of every bond. The primary antibodies (dilution) were incubated, as follows: Hsp70 (abcam, 1/1000), caspase 3 (abcam, 1/1000), cytochrome c (abcam, 1/1000), β-actin (Mouse, Sigma) (1/1000), followed by secondary antibody incubation.

### 1.19 H&E staining.

Heart, liver, spleen, lung and kidney of tumor-bearing mice and normal mice in each group, tumor tissue of tumor-bearing mice in each group were all excised and fixed in 10% formaldehyde and embedded in paraffin and stained with hematoxylin and eosin (H&E) to confirm histology.

#### 1.20 ICP-MS analysis.

The sample preparation for the ICP-MS analysis was as follows. Briefly,  $1 \times 10^5$  cells (A549 cells and MRC-5 cells) were treated with 60 µg/mL CyPT-AuNRs for 3h. Then the cells were digested by aqua regia and the final sample solutions were diluted to a fixed volume (10 ml). Each treatment was repeated for 3 times. The mass of gold element determined from ICP-MS was compared.

# 2. Synthetic Procedures and Characterization Details of Cy-PT and CyPT-AuNRs



Scheme S1. Synthetic Approaches of CyPT. a) 1,3-dibromopropane,  $K_2CO_3$ , acetone, refluxed for 12 h, 92%; b) acetonitrile, refluxed for 12 h, 90%; c) DMF,  $CH_2Cl_2$ ,  $POCl_3$ , 45 °C, 3 h, 85%; d) n-Butyl alcohol:benzene = 7:3 (v/v), refluxed, 3 h, 70%.

**Synthesis of Compound 1**: Methyl 4-hydroxybenzoate (152 mg, 1 mmol) and 1,3-dibromopropane (200 mg, 1 mmol) were dissolved in dry acetone (250 mL), then anhydrous potassium carbonate (275 mg, 2 mmol) was added. The mixture was stirred under a dry argon atmosphere for 12 h and monitored by TLC. After the reaction was finished, the mixture was cooled to room temperature, evaporated under reduced pressure and partitioned with CH2Cl2 and saturated KBr solution. Finally, the organic layer was separated. Purification by column chromatography on silica eluting with CH2Cl2/EtOAc (8:1) gave the product compound 1 as white powder (248.1 mg, yield: 92%).

**Synthesis of Compound 2**: 1,1,2-trimethyl-1H-benz[e]indole (2 g, 10 mmol) and compound 1 (2.7 g, 10 mmol) were mixed in 10 mL anhydrous acetonitrile in 50 mL round flask, then the mixture was refluxed for 12 h, then stopped heating and cooled down. The precipitate was filtered through a Buchner funnel, and the solid product was washed by diethyl ether and dried in vacuum to afford dark violet product (4.32 g, yield: 90%).

**Synthesis of Compound 3**: A solution of 40 mL of anhydrous N, N-dimethylformamide (DMF) and 40 mL of anhydrous CH2Cl2 was placed in a 250 mL round-bottom flask, chilling the solution to -10 °C and then stirring for 20 min. Phosphorus oxychloride (37 mL), with 35 mL of anhydrous CH2Cl2 was dropwise added into above

solution through a constant pressure drop of liquid funnel. 4-(4-Hydroxyphenyl)cyclohexanone (10 g, 52.6 mmol) was added into the mixture in batches, the solution changed from colorless into yellow immediately. Then the solution was slowly heated to 45 °C for 3 h, then cooled down, poured into a lot of ice, and allowed to stand overnight. The yellow solid was collected through a buchner funnel and dried in vacuum (12.9 g, yield: 85%). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 500 MHz)  $\delta$  (ppm): 9.22 (s, 1H), 8.12 (s, 1H), 8.08 (s, 1H), 7.65-7.34 (m, 2H), 7.14 (s, 1H), 6.69-6.64 (m, 2H), 2.38-2.71 (m, 1H), 2.53-2.45 (m, 2H), 2.28-2.26 (m, 2H). <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 125 MHz)  $\delta$  (ppm): 191.8, 162.9, 155.7, 148.5, 145.7, 142.4, 131.2, 127.6, 127.5, 117.8, 115.1, 37.2, 31.7, 30.6. LC-MS (ESI-): m/z C<sub>14</sub>H<sub>13</sub>ClO<sub>3</sub> calcd. 264.0553, found [M-H]<sup>-</sup> 263.0481.

**Synthesis of Compound CyPT**: Compound 2 (0.96 g, 2 mmol) and 3 (0.26 g, 1 mmol) were resolved in 100 mL mixed solution of n-butyl alcohol and benzene (7:3, v/v) in 250 mL round flask, refluxed for 3 h, dried in vacuum, to obtain green solid. The crude product was purified by silica gel chromatography using EtOAc/CH<sub>3</sub>OH (8:1, v/v) as eluent to afford **CyPT** as green solid (0.651 g, 70% yield). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 500 MHz)  $\delta$  (ppm): 9.37 (s, 1H), 8.41-8.04 (m, 8H), 7.83-7.77 (m, 5H), 7.69-7.51 (m, 2H), 7.25-7.23 (m, 2H), 6.85-6.83 (m, 5H), 6.39-6.36 (m, 2H), 4.51-4.50 (m, 3H), 4.14 (s, 4H), 3.80 (s, 7H), 3.04-3.01 (m, 2H), 2.55-2.51 (m, 6H), 2.24 (m, 2H), 1.97-1.92 (m, 11H), 1.56-1.22 (m, 4H). <sup>13</sup>C NMR (DMSO-d<sub>6</sub>, 125 MHz)  $\delta$  (ppm): 174.2, 166.2, 162.3, 156.7, 147.6, 142.7, 140.1, 135.3, 134.1, 131.9, 131.6, 130.9, 130.4, 128.6, 128.2, 127.8, 126.5, 125.5, 122.7, 122.4, 115.7, 114.9, 114.7, 112.1, 101.6, 65.2, 52.2, 51.2, 41.6, 40.5, 40.4, 40.3, 40.2, 40.1, 39.9, 39.8, 39.6, 39.4, 37.8, 33.9, 27.5, 27.2. LC-MS (ESI<sup>+</sup>): m/z C<sub>66</sub>H<sub>64</sub>ClN<sub>2</sub>O<sub>7</sub><sup>+</sup> calcd. 1031.45, found [M<sup>+</sup>] 1031.69.

Preparation of CyPT-AuNRs: The AuNRs were synthesized in an aqueous solution using a seed-mediated growth method. The 3-4 nm gold seed particles were prepared by mixing 5 mL of 0.5 mM HAuCl<sub>4</sub> with 5 mL of 0.2 M CTAB. The solution was stirred vigorously followed by drop wise addition of 600 mL freshly prepared icecold 10 mM of NaBH<sub>4</sub>. In the AuNRs synthesis, 18 mL of 5 mM HAuCl<sub>4</sub> and 225 mL of 0.1 M AgNO<sub>3</sub> were added to 90 mL of 0.2 M CTAB and then 200 mL of 1.2 M HCl and 10.5 mL of 10 mM ascorbic acid were added and gently swirled as the color changed from dark orange to colorless. After the color had changed, 120 mL of the CTAB-stabilized gold seed solution was rapidly injected. The resulting solution was gently mixed for 10 s and left undisturbed overnight. Finally, the AuNRs solution was centrifuged at 12,000 rpm for 15 min to stop the reaction. The supernatant was removed and precipitate was resuspended in ultrapure water. Then, 20 mM 2mercaptoethanol was added to 50 mL prepared AuNRs solution, the the mixture was stirred overnight. The mixture solution was centrifuged at 12,000 rpm for 15 min three times to stop the reaction and removed the excessive 2-mercaptoethanol, the AuNRs-OH was obtained. AuNRs-OH resuspended in anhydrous ethanol for the next experiment. 2,2-Dithiodiglycolic acid (20 mM) was dissolved in anhydrous ethanol, then 20 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), 20 mM 4-dimethylaminopyridine (DMAP) and 1hydroxybenzotriazole (HOBt) (catalytic) were added to above solution dissolved for 1 h, next the prepared AuNRs-OH solution was dropwise added into above mixture solution through a constant pressure drop of liquid funnel reacted for 24 h. The AuNRs-S-S was collected through centrifuged at 12,000 rpm for 15 min three times and resuspended in anhydrous ethanol. Next, 20 mM EDC, 20 mM DMAP and HOBt (catalytic) were resolved in the prepared AuNRs-S-S solution and stirred for 1 h. Then the Cy-PT that was synthesis in Support Information was dropwise added into above mixture solution through a constant pressure drop of liquid funnel reacted for 24 h. The final product CyPT-AuNRs was obtained after centrifuged at 12,000 rpm for 15 min three times to stop the

reaction and removed the excessive Cy-PT. CyPT-AuNRs was resuspended in ultrapure water and store at 4 °C.

# 3. Quantitative analysis of CyPT on the surface of AuNRs



Figure S1. a: UV-vis absorption spectra of CyPT at different concentrations. b: Calibration curve (Absorbance at 830 nm against the concentration of CyPT) for quantification of CyPT.

#### 4. Effect of pH Values on CyPT-AuNRs

As a starting point, it is necessary to understand the pH effect on the potential fluorescence behaviour of our nanoprobe CyPT-AuNRs in the absence and presence of GSH (HEPES buffer solution 10 mM). The results demonstrate that fluorescence intensity of CyPT-AuNRs shows pH-dependent property over the pH range from 3.0-9.0 (Figure. S1). After the probe was incubated with GSH for 20 min, the fluorescence intensity stayed at high level. When pH ranges from 7.0 to 8.0, the fluorescence intensity of CyPT-AuNRs with GSH is still 6-7 fold higher than that of only CyPT-AuNRs, which indicates that our nanoprobe CyPT-AuNRs would be suitable to be applied in living cells in which pH ranges from 5.0 to 8.0.



Figure S2. Effect of pH values on CyPT-AuNRs. pH ranges from 3.0 to 9.0.

# 5. Selectivity to GSH

To verify the fluorescent response to other biological analytes, we tested the selectivity of CyPT-AuNRs against physiological relevant reactive oxygen species (ROS) and reactive nitrogen species (RNS) in HEPES solution (10 mM, pH 7.4). Compared to other ROS and RNS, CyPT-AuNRs offered remarkable fluorescent response for GSH. Figure S2 demonstrated that H<sub>2</sub>O<sub>2</sub>, methyl linoleate hydroperoxide, cumene hydroperoxide, tert-butyl hydroperoxide, NO, ClO<sup>-</sup>, S-nitrosoglutathione (GSNO), 'OH and ONOO<sup>-</sup> caused almost no response in emission signal for 20 min incubation. The results showed that CyPT-AuNRs had good selectivity for GSH detection over other biologically relevant ROS and RNS. The fluorescence responses of CyPT-AuNRs to other reactive species sulfur (RSS) were also evaluated (Figure S2). CyPT-AuNRs (60 µg/mL) could provide obviously

fluorescence response to  $H_2S_n$ , such as  $H_2S_2$ ,  $H_2S_4$ ,  $H_2S_n$  (derived from  $H_2S$  and  $S_8$ ). There was also obtained limit interference after addition of  $H_2S$  and cysteine hydropersulfide. However, no obvious changes in spectra were observed upon the addition of  $S_8$ , PhCH<sub>2</sub>S<sub>4</sub>CH<sub>2</sub>Ph, Cys-polysulfide, GSH, Cys, Hcy, Cystine, and GSSG. The addition of ascorbic acid and  $\alpha$ -tocopherol also caused no interferences. These results demonstrated that CyPT-AuNRs was a highly selective fluorescent probe for GSH detection over other RSS and physiologically relevant reduced species. Moreover, CyPT-AuNRs had good fluorescence stability in HEPES buffer ranging from pH 4.0 to 9.0. Taken together, these kinetic and selectivity assays revealed that our probe could work well under physiological conditions for the investigation of GSH.



Figure S3. The selectivity of CyPT-AuNRs to GSH. 1. GSH, 2.  $H_2O_2$ , 3. methyl linoleate hydroperoxide, 4. cumene hydroperoxide, 5. tert-butyl hydroperoxide, 6. NO, 7. ClO<sup>-</sup>, 8. GSNO, 9. OH, 10. ONOO<sup>-</sup>, 11.  $H_2S_2$ , 12.  $H_2S_4$ , 13.  $H_2S_n$ , 14.  $S_8$ , 15. PhCH<sub>2</sub>S<sub>4</sub>CH<sub>2</sub>Ph, 16. Cys-polysulfide, 17. Cys, 18. Hcy, 19. Cystine, 20. GSSG, 21. ascorbic acid, 22.  $\alpha$ -tocopherol.





Figure S4. The bright field of cells in Figure 2. Scale bar =  $10 \mu m$ .

# 7. The Mean Fluorescence Intensity of Figure 2b.



Figure S5. The mean fluorescence intensity of Figure 2b.

8. GSH Concentration in A549 Cells and MRC-5 Cells.



Figure S6. GSH concentration in A549 cells and MRC-5 cells by total glutathione assay kit.

# 9. Comparison of CyPT-AuNRs Content in A549 Cells and MRC-5 Cells.



Figure S7. Comparison of CyPT-AuNRs content in A549 cells and MRC-5 cells by ICP-MS.

# 10. The Bright Field of Cells in Figure 3



Figure S8. The bright field of cells in Figure 3. Scale bar =  $10 \mu m$ .

# 11. NIR Fluorescent Imaging of Mice and Tumor-bearing Mice

Encouraged by the results that the detection of GSH used CyPT-AuNRs in living tumor cells, we next strived to investigate the detection of GSH in mice and tumor-bearing mice benefit from NIR fluorescence emission of CyPT-AuNRs. First, the application of CyPT-AuNRs for fluorescence imaging in vivo was studied in BALB/c mice. Simultaneously, pharmacokinetics of CyPT-AuNRs in vivo also was investigated. All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals, and protocols were approved by the Institutional Animal Care and Use Committee in Binzhou Medical University, Yantai, China (Approval Number: No.BZ2014-102R). CyPT-AuNRs (5 mg/kg) was injected into BALB/c mice by intravenous (i.v.) injection. NIR fluorescent imaging was performed at different time points by Perkinelmer IVIS Lumina XRMS Series III In Vivo Imaging System. As shown as Figure S6a, a weak fluorescence signal was obtained at the beginning, then the fluorescence signal was becoming stronger and stronger with the passage of

time. The strongest fluorescence signal was obtained after CyPT-AuNRs was injected 12 h, next the fluorescence signal was getting weaker due to the metabolism of mice. And the main tissue and organ were removed by dissected, the fluorescence signal of ex vivo organs (heart, liver, spleen, lung, kidney) were analyzed by fluorescence imaging. High fluorescent contrast between liver and other normal tissue in BALB/c mice was observed, suggested not only that the amount of GSH in vivo turn the fluorescence on, but also the liver preferential accumulation of CyPT-AuNRs due to the liver is a major metabolic site. To further research the pathway of excreted CyPT-AuNRs, the urine of all the mice at different time points after injection was collected. As shown in Figure S7, the fluorescence signal was decrease with time. The results revealed that the nanoprobe CyPT-AuNRs not only could detect the GSH in deep organization in vivo, but also had an efficient renal clearance to avoid the long-term risk of adverse effects.

Nanoparticles can preferentially accumulate in the tumor site through enhanced permeability and retention (EPR) effect without requiring chemical conjugation of tumor-targeting ligands have been recently identifed and have shown unique properties for cancer in vivo imaging. In order to evaluate the cancer specificity of CyPT-AuNRs, nude mice bearing A549 cell xenografts were established as described previously. CyPT-AuNRs (5 mg/kg) intravenously into the nude mice bearing A549 cell tumor xenografts and then NIR fluorescent imaging was performed by In Vivo Imaging System. As shown as Figure S6b, the fluorescence signal appeared in the location of liver after intravenous injection 0.5 h, and then CyPT-AuNRs was injected after 6 h, a strong fluorescence signal was obtained in the tumor tissue site. As our expected, the fluorescence signal in tumor site was continuous enhanced within 48 h. We reasoned that CyPT-AuNRs was mainly accumulated in tumor by the EPR effect and the tumor site has a higher concentration of GSH. Then the effective fluorescence signal was still existed after 72 h. To further confirm the accumulation of CyPT-AuNRs in tumor tissue site, the mice were sacrificed and their main organs including tumors were dissected. The ex vivo NIR imaging of the dissected organs from above models further confirmed its tumor preferential accumulation (Figure S6b). The result demonstrated that CyPT-AuNRs was an effective nano-reagent for tumor-targeted in vivo. And the long-time accumulation of CyPT-AuNRs in tumor site was beneficial to the efficient of PDT and PTT.



Figure S9. a) CyPT-AuNRs for fluorescence imaging in BALB/c mice and ex vivo fluorescent imaging of dissected main organs and the mean fluorescence intensity of liver in different times. b) CyPT-AuNRs for fluorescence imaging in subcutaneous A549 tumor model and ex vivo fluorescent imaging of dissected main organs and the mean fluorescence intensity of tumor in different times. He: heart; Li: liver; Sp: spleen; Lu: lung; Ki: kidney; Tu: tumor. Images displayed represent emission intensities collected window: 830 - 900 nm,  $\lambda ex = 810$  nm. Data are presented as mean  $\pm$  SD (n = 5).

# 12. Fluorescence of Urine of the Mice at Different Time Points



Figure S10. The fluorescence of urine of the mice at different time points after injected CyPT-AuNRs.

13. The Histogram of Temperature in Figure 5a.



Figure S11. The histogram of temperature in Figure 5a.

# 14. <sup>1</sup>H NMR, <sup>13</sup>C NMR and LC-MS of CyPT <sup>1</sup>H NMR



<sup>13</sup>C NMR



# LC-MS





# 15. References

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