Supporting Information

For

Near Infrared Light-Actuated Gold Nanorods with Cisplatin-Polypeptide Wrapping for Targeted Therapy of Triple Negative Breast Cancer

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Materials and Methods

1. Materials

Chloroauric acid (HAuCl₄·3H₂O), cetyltrimethyl ammonium bromide (CTAB), sodium borohydride (NaBH₄), silver nitrate (AgNO₃), L-ascorbic acid (AA), Rhodamine B (RhoB), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDCI), 1-hydroxybenzotrizole (HOBT), Tris(2-carboxyethyl) phosphine (TCEP), FITC-labeled dextran (FITC-Dex, *Mw* 2,000 kDa) and folic acid (FA) were all obtained from Sigma-Aldrich (Shanghai, China). Methoxyl poly(ethylene glycol) amine (mPEG₁₁₄-NH₂), maleimide poly(ethylene glycol) amine (Mal-PEG₁₁₄-NH₂) and amine poly(ethylene glycol) amine (H₂N-PEG₁₁₄-NH₂) were all purchased from Jenkeam Technology Co. Ltd. (Beijing, China). Cisplatin (Pt) was purchased from Platinum Energy. Co. Ltd (Shandong, China) and aquated according to a literature method.[20] Hoechst 33342, Calcein-AM and ethidium bomodimer-1 (EthD-1) were purchased from Life Technology (Carlsbad, CA). The RPMI1640 medium, fetal bovine serum (FBS), penicillin-streptomycin solution and trypsin-EDTA solution were purchased from Gibco (Tulsa, OK). Dialysis tubes with molecular weight cut off (MWCO) of 3.5 kDa or 7~10 kDa were obtained from MyFil Biotechnology Co. Ltd (Shanghai, China). Ultrapure water was produced using a Milli-Q water purification system. All other chemicals if not mentioned were analytical grade and obtained from SinoPharm Chemical Reagent Co., Ltd. (Shanghai, China).

2. Synthesis of thiolated mPEG-g-PGA graft copolymer
To synthesize mPEG-g-PGA graft copolymer with pendent thiol groups, PGA homopolymer was firstly randomly grafted with mPEG_{5k}-NH_{2}, and then conjugated with 2-aminoethyl 2-pyridyl disulfide. Briefly, 5.95 µmol of PGA_{130} was dissolved in 8 mL of DMSO with addition of 0.77 mmol of EDCI and 0.77 mmol of HOBT. The mixture was reacted for 2 h, and then added with 1.79×10^{-2} mmol of mPEG_{5k}-NH_{2}, the reaction was continued for 24 h at room temperature, followed by dialyzing against DI water and lyophilizing. The resultant mPEG-g-PGA graft copolymer was conjugated with 2-aminoethyl 2-pyridyl disulfide at a monomer to copolymer molar ratio of 13. Typically, 5.0 µmol of mPEG-g-PGA was dissolved in 8 mL of DMSO with the addition of 0.65 mmol of EDCI and 0.65 mmol of HOBT. Two hours later 0.065 mmol of 2,2'-dipyridyl disulfide was added, and the reaction was continued for 24 h at RT. mPEG-g-PGA-SH copolymer was obtained after reducing with TCEP in phosphor buffer at pH 7.0, dialyzing against DI water and lyophilizing. The successful thiolation of PGA backbone was confirmed by $^1$H-NMR examination.

3. RhoB labeling of mPEG-g-PGA graft copolymer (RhoB-PEG-g-PGA)

To synthesize Rho-B labeled PGA, 9.58 mg of RhoB (0.02 mmol) was dissolved in 10 mL of DMAC and then its carboxyl group was pre-activated with 57.5 mg of EDCI (0.03 mmol) and 40.5 mg of HOBT (0.03 mmol) for 2 h. Afterwards, the above solution was added slowly to 10 mL of DMAC solution containing 100 mg of NH_{2}-PEG_{5k}-NH_{2} (20 µmol). The reaction was continued for 24 h at room temperature and then excess RhoB was removed by dialyzing against DI water. To conjugate RhoB-PEG_{5k}-NH_{2} onto PGA backbone, 50 mg of PGA (3.0 µmol), 95.5 mg of EDCI (0.5
mmol) and 52.5 mg of HOBT (0.5 mmol) were co-dissolved in 5 mL of DMAC to activate the carboxyl group of PGA. Afterwards, 50 mg of RhoB-PEG$_{5k}$-NH$_2$ (9.0 μmol) was added and the reaction was continued for 24 h. The unreacted PEG was removed by dialyzing against DI water (MWCO 7-10 k Da), and final product was obtained as pink powder after lyophilizing. The successful conjugation of RhoB-PEG onto PGA backbone was confirmed by $^1$H-NMR examination.

4. Synthesis of FA conjugated 2-aminoethyl 2-pyridyl disulfide (FA-S-S-pyridine)

Typically, 0.22 g of FA (0.5 mmol), 115 mg of EDC·HCl (0.6 mmol) and 81 mg of HOBT (0.6 mmol) were co-dissolved in 35 mL of anhydrous DMAC under sonication. The reaction was continued for 2 h. Afterwards, 5 mL of DMAC solution containing 0.55 mmol of 2-aminoethyl 2-pyridyl disulfide and 150 uL of TEA was slowly added to above solution in 1 h. The reaction was continued for 24 h and precipitated with ether. The precipitate was vacuum-dried to obtain 270 mg of yellow powder with a yield of 88%. The chemical structure and molecular weight the final product was confirmed by $^1$H-NMR and ESI mass examinations.

5. Synthesis of FA conjugated PEG-g-PGA graft copolymer bearing pendent thiol groups (FA-PEG-g-PGA-SH)

Firstly, 5.95 μmol of PGA$_{130}$ was dissolved in 8 mL of DMSO, and the carboxyl groups were activated with 0.77 mmol of EDCI and 0.77 mmol of HOBT for 2 h. Afterwards, 17.9 μmol of Mal-PEG$_{5k}$-NH$_2$ was added, and the reaction was continued for 24 h at room temperature. Mal-PEG-g-PGA copolymer was obtained after dialysis against DI water and lyophilizing. Secondly, 12.2 mg (0.02 mmol) of FA-S-S-pyridyl
was firstly reduced with 20 mg of TCEP in 5 mL of DMAC solution for 2 h, the mixture was then added to PB (pH 7.0) solution containing 318 mg of Mal-PEG-g-PGA (1x10^{-2} mmol), the reaction was continued for 4 h. Afterwards, the mixture was dialyzed against DI water until complete removing of excess FA as monitored using UV-Vis spectrophotometer. Finally, FA-PEG-g-PGA copolymer with pendent thiol groups was synthesized by conjugating 2-aminoethyl 2-pyridyl disulfide onto the backbone of FA-PEG-g-PGA. Typically, 5.0x10^{-3} mmol of FA-PEG-g-PGA was dissolved in 8 mL of DMSO with the addition of 0.65 mmol of EDCI and 0.65 mmol of HOBT. Two hours later, 0.065 mmol of 2,2'-dipyridyl disulfide was added, and the reaction was continued for 24 h at room temperature. The final product was obtained after reduced with TCEP in phosphor buffer at pH 7.0, dialyzed against DI water and lyophilized. The successful thiolation of PGA backbone was confirmed by 1H-NMR examination.

6. Preparation and characterization of GNR@Pt nanoparticles.

PGA homopolymer was firstly grafted with poly(ethylene glycol) amine with or without FA conjugation. The resultant FA-PEG-g-PGA or mPEG-g-PGA graft copolymers were then functionalized with pendent thiol groups (see Supporting Information for synthesis and characterization). CTAB-protected GNRs were then self-assembly coated with mPEG-g-PGA-SH. Typically, 1 mL of freshly prepared CTAB-GNR solution containing 2.95 mM of Au was centrifuged for 10 min at 8000 rpm, the pellet was redispersed in DI water and mixed with 1 mL of aqueous solution of mPEG-g-PGA-SH (13 mg/mL, 0.39 mM). The reaction was continued overnight at room temperature under constant shaking. The resultant GNR-PGA nanoparticles
were thoroughly washed by DI water for several times to remove excess mPEG-g-PGA-SH copolymer and CTAB by centrifugation at 8000 rpm. FA-conjugated or RhoB-labeled GNRs were prepared by coating CTAB-GNR nanoparticles with FA-PEG-g-PGA-SH or RhoB-PEG-g-PGA-SH copolymer.

To prepare cisplatin-loaded GNR@Pt nanoparticles, 1 mL of GNR-PGA suspension containing 2.95 mM of Au was mixed with 1 mL aqueous solution of 1.1 mM aquated cisplatin under gently stirring, the reaction was continued overnight at room temperature. The prepared GNR@Pt was purified by water washing and centrifugation at 8000 rpm. The Pt to Au molar ratio in the resultant GNR@Pt hybrid nanoparticles was measured using ICP-MS. The stability of GNR@Pt nanoparticles in water, 10 mM PB or complete FBS solution was measured using UV-Vis spectroscopic measurement. The successful coating of mPEG-g-PGA-SH copolymer and loading of cisplatin on GNRs was monitored by UV-Vis spectroscopic and surface charge measurement, respectively. The morphology of the resultant GNR@Pt nanoparticles was examined by TEM measurement. The laser irradiation induced temperature elevation of GNR@Pt suspension was recorded using an infrared camera thermographic system (IRTech, Shanghai, China). A portable 655 nm diode laser was used as the photo source (Changchun New Industries Optoelectronics Tech. Co., Ltd, Changchun, China). To investigate chloride ion-triggered cisplatin release from GNR@Pt nanoparticles, 100 μL of aqueous suspension of GNR@Pt was dispersed in 0.9% (w/v) saline solution, the mixture was then stored at 37°C incubator under constant shaking. The mixture was centrifuged at different time intervals to collect the residual GNR@Pt nanoparticles, and the amount of cisplatin released was determined by measuring the fraction of cisplatin remained on GNR@Pt nanoparticles using ICP-MS measurement.
7. Cell lines and cell culture.

Murine 4T1 breast cancer cells were obtained from cell bank of Chinese Academy of Sciences, Shanghai, China. 4T1 cells with stable luciferase expression (4T1-Luc) were produced by lentiviral vector (encoded with a pGL3 luciferase promoter) mediated transfection. Both 4T1 and 4T1-Luc cells were routinely cultured in complete RPMI 1640 cell culture medium containing 10% fetal bovine serum (FBS), 2.5 g·L\(^{-1}\) of glucose, 0.11 g·L\(^{-1}\) of sodium pyruvate, 100 U·mL\(^{-1}\) of penicillin G sodium and 100 μg·mL\(^{-1}\) of streptomycin sulfate. The cells were maintained in incubators at 37 °C under a humidified atmosphere containing 5% CO\(_2\). All experiments were performed on cells in the logarithmic phase of growth.

8. Cellular uptake and intracellular distribution of GNR@Pt nanoparticles.

4T1 cells were seeded onto 12-well plates at a density of 5 × 10\(^4\) cells per well. The cells were then incubated with RhoB-labeled FA-GNR@Pt or GNR@Pt nanoparticles (termed as FA-GNR@Pt-RhoB or GNR@Pt-RhoB) at Au concentration of 105 μM or cisplatin concentration of 5.3 μM, respectively. The cellular fluorescence intensity of RhoB was analyzed using a Beckman-Coulter flow cytometer at desired time intervals. Background and auto-fluorescence was determined using mock-treated cells. Data analysis was carried out using the FCS Express 3 software (De Novo Software, CA, USA). The intracellular distribution of GNR@Pt-RhoB nanoparticles was measured using CLSM and TEM examination, respectively. The cell samples for TEM measurement were prepared by following the procedure we described previously.\(^{63}\)

9. Chemo- and phototoxicity of GNR@Pt nanoparticles \textit{in vitro}.

The chemotoxicity of GNR@Pt or FA-GNR@Pt nanoparticles was measured using MTT assay. Briefly, 4T1 cells were seeded in a 96-well plate at a density of 3500 cells per well in 100 μL of medium. After 24 h pre-incubation, the cells were
incubated with FA-GNR@Pt, GNR@Pt or cisplatin at different cisplatin concentrations for 4 h or 24 h. The cell viability was evaluated by MTT assay at the desired time intervals, which was expressed as relative cell viability by normalized with that of the untreated cells controls. To compare the cytotoxicity of CTAB-GNRs, covalently wrapped GNRs (GNR-PGA) and electrostatically coated GNR@PGA nanoparticles, 4T1 cells were treated with three kinds of GNR formulations for 24 h. The cell viability was then evaluated by MTT assay.

To quantitatively determine the phototoxicity of GNR@Pt in 4T1 cells, the cells were pre-incubated with FA-GNR@Pt, GNR@Pt, FA-GNR, GNR or cisplatin for 4 h or 24 h at a cisplatin concentration of 5.3 μM or Au concentration of 105 μM, respectively. Afterwads, the cells were irradiated with 655 nm laser for 2, 4 or 6 min at a light intensity of 2.0 W/cm². After additional 24 h incubation, the cell viability was measured by MTT assay. To elucidate the synergistic effect between chemotoxicity of cisplatin and hyperthermia effect of GNR in vivid, 4T1 cells were seeded onto a 12-well plate at a density of 5x10^4 cells per well and incubated with FA-GNR@Pt, GNR@Pt, FA-GNR, GNR or cisplatin at an equal cisplatin concentration of 5.3 μM or Au concentration of 105 μM, respectively. After 4 h nanoparticle incubation, the excess medium was removed and cells were washed twice to remove any free GNRs attached on the cell surface. Next, the cells were irradiated with 655 nm laser for 3 min at a photo intensity of 2.0 W/cm². The cells were then stained with Calcein-AM, Ethidium homodimer-1 and Hoechst 33342 to distinguish the live, dead and apoptotic cells respectively by following a procedure described previously. The fluorescence pattern of the stained cells were examined using an invert fluorescence microscopy (Olympus, 81X, Japan).

10. In vivo pharmacokinetics and biodistribution of GNR@Pt nanoparticles.
To investigate the blood circulation of GNR@Pt nanoparticles, Balb/c nude mice (female, 4-5 weeks, 18 ± 2 g, Shanghai Experimental Animal Center, Shanghai, China) were i.v. injected with 100 μl PB solution of FA-GNR@Pt, GNR@Pt or cisplatin at an equal cisplatin dose of 1.0 mg/kg or GNR dose 13.7 mg/kg. Blood samples were collected at 5 min, 30 min, 1 h, 2 h and 24 h time intervals post i.v. injection. The blood concentration of platinum and Au were then quantitatively examined by ICP-MS analysis. To examine the biodistribution of GNR@Pt nanoparticles in vivo, each Balb/c nude mouse was injected with $1 \times 10^6$ 4T1 cells on the right mammary gland. Seven days later, mice bearing 4T1 tumors of 200 mm³ were grouped (n = 3) and intravenously injected with FA-GNR@Pt, GNR@Pt or cisplatin suspension at an equal GNR dose of 6.9 mg/kg or cisplatin dose of 0.5 mg/kg. The mice were sacrificed 2 h or 24 h post nanoparticle administration to collect the major organs (heart, liver, spleen, lung and kidney) and tumors. The organ distribution of GNR and cisplatin was determined by ICP-MS analysis.

11. Hyperthermia effect in vivo and photothermal ablation of peripheral tumor blood vessels.

Nude mice bearing 4T1 orthotopic tumors were used to evaluate the hyperthermia effect of GNR@Pt nanoparticles in vivo. All animal procedures were carried out under the guidelines approved by the Institutional Animal Care and Use Committee of the Shanghai Institute of Material Medica, Chinese Academy of Sciences. Briefly, 200 μL suspension of GNR-PGA, GNR@Pt or FA-GNR@Pt was i.v. injected into nude mouse bearing 100 mm³ 4T1 tumor (at an equal GNR dose of 6.9 mg/kg or cisplatin dose of 0.5 mg/kg), the tumor was irradiated with 655 nm laser at photo density of 2.0 W/cm² at different time intervals post nanoparticle injection. Near infrared imager was used to record the temperature change curve. To demonstrate the
ability of GNR@Pt to eliminate the peripheral tumor blood vessels, orthotopic 4T1 tumor was established by injecting $1 \times 10^6$ 4T1 cells on the right mammary gland of Balb/c nude mouse (female, 4-5 weeks, 18 ± 2 g, Shanghai Experimental Animal Center, Shanghai, China). Three days later, mice bearing 4T1 tumors of 50 mm$^3$ were randomly grouped ($n = 3$) and injected through tail vein with 200 µL of GNR@Pt suspension at an equal Au dose of 6.9 mg/kg or cisplatin dose of 0.5 mg/kg, respectively. Two hours later after injection, the tumors were locally irradiated by 655 nm NIR laser at a photo density of 2.0 W/cm$^2$ for 2 mins. Twenty four hours post nanoparticles injection, FITC-Dextran was injected intravenously to the tumor-bearing mice at a dose of 50 mg/kg and then the green-fluorescent adherent to the capillary was recorded to manifest the density of capillary surrounding the tumor using a Cellvizio confocal laser endomicroscopy system (Cellvizio®, F-400, Mauna Kea Technologies, French).

12. Anti-tumor efficacy of FA-GNR@Pt nanoparticles administrated through tail vein or intratumoral injection

The anti-tumor efficacy of FA-GNR@Pt nanoparticles administrated through intratumoral injection was evaluated using a 4T1-Luc orthotopic tumor model. All animal procedures were carried out under the guidelines approved by the Institutional Animal Care and Use Committee of the Shanghai Institute of Material Medica, Chinese Academy of Sciences. Each Balb/c nude mouse was injected with $1 \times 10^6$ 4T1-Luc cells on the right mammary gland. Three days later, the mice bearing 4T1-Luc tumors of 50 mm$^3$ were randomly grouped ($n = 6$), i.v. injected with 200 µL suspension of GNR-PGA, GNR@Pt or FA-GNR@Pt (GNR dose of 6.9 mg/kg or cisplatin dose of 0.5 mg/kg), or intratumorally injected with 50 µL of FA-GNR@Pt suspension or other formulations at an equal GNR dose of 1.7 mg Au/kg or cisplatin.
dose of 0.13 mg/kg, respectively. Two hours later after injection, the GNR-PGA, GNR@Pt and FA-GNR@Pt groups were selectively irradiated by 655 nm NIR laser at an energy density of 1.0 W/cm² for 2 mins (Changchun New Industries Optoelectronics Tech. Co., Ltd, Changchun, China). The tumor volume was monitored with a digital caliper and the body weights were examined with an electronic balance, respectively. All mice were sacrificed 25 days later after first injection. All tumors and lungs were collected for weighting, photo imaging and histological examination. The tumor volume was calculated by formula $V = L \times W \times W/2$ (L, the longest dimension; W, the shortest dimension).

To investigate lung metastasis of 4T1 cancer cells from the primary tumor, the mice were injected with 100 ul D-luciferin (20mg/ml) and anesthetized with isoflurane at the end of therapeutic period. The whole body BLI images were collected with an IVIS imaging system (Xenogen, Alameda, CA) 10 mins post D-luciferin injection. For BLI imaging of lung tissue, lungs from the same mouse group were immersed in D-luciferin (5.0 mg/mL) for 5 mins and then imaged with the IVIS imaging system. Quantitative luminescence intensity analysis was performed with the Live Image software (Xenogen, Alameda, CA). All major organs (tumors, heart, liver, spleen, lung and kidneys) were harvested and fixed in 10% formalin solution, dehydrated, sliced into 5.0 µm sections and subjected to H&E staining assay.

13. Liver function examination

To investigate the biosafety of GNR@Pt, GNR-PGA and cisplatin, Balb/c mice (n=3) were intravenously injected with PBS, GNR@Pt, GNR-PGA or cisplatin suspension at an equal GNR dose of 6.8 mg/kg or cisplatin dose of 0.5 mg/kg. Blood samples were collected 24 h later after nanoparticle injection and evaluated using blood biochemical analysis. The liver function was evaluated with serum levels of alanine
aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin level (T-BIL), total protein (TP), albumin (ALB), globulin (GLOB), alkaline phosphatase (ALP), ALB/GLOB and AST/ALT.
Table SI 1. The influence of intravenously injected cisplatin, GNR-PGA and GNR@Pt on the liver function examined using blood biochemical analysis. The serum levels of albumin (ALB), globulin (GLOB), and ALB/GLOB (A/G), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin level (TBIL) and total protein (TP) were examined, respectively.

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<th>Formulations</th>
<th>PBS</th>
<th>GNR-PGA</th>
<th>Cisplatin</th>
<th>GNR@Pt</th>
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<td>A/G</td>
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<td>0.61±0.005</td>
<td>0.67±0.01</td>
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<td>GLOB (g/L)</td>
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<td>ALB (g/L)</td>
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<td>11.1±0.3</td>
<td>12.4±0.05</td>
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<tr>
<td>ALP (U/L)</td>
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<td>4.6±4.1</td>
<td>12.5±1.4</td>
<td>4.45±3.4</td>
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<tr>
<td>ALT (U/L)</td>
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<td>19.75±5.0</td>
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<td>17.6±1.9</td>
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<tr>
<td>AST (U/L)</td>
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<td>T-BIL (μmol/L)</td>
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<td>TP (g/L)</td>
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<td>29.4±0.70</td>
<td>30.9±0.50</td>
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Figure SI 1. Synthetic routes for thiolated mPEG-g-PGA graft copolymer.
Figure SI 2. $^1$H-NMR spectra of mPEG-g-PGA-S-S-Pyridine (Py), mPEG-g-PGA graft copolymer and 2-aminoethyl 2-pyridyl disulfide (NH$_2$-S-S-Py), respectively.
Figure SI 3. Synthesis route for FA-S-S-pyridine.

Figure SI 4. ESI-MS spectrum of FA-S-S-pyridine.

Figure SI 5. Synthetic route for FA conjugated PEG-g-PGA-SH copolymer.
Figure SI 6. $^1$H-NMR spectra of FA-PEG-g-PGA, Mal-PEG-g-PGA graft copolymer and FA, respectively.
Figure SI 7. UV-Vis spectra of FA-PEG-g-PGA, Mal-PEG-g-PGA and FA, respectively. The presence of the absorption peak of FA in FA-PEG-g-PGA copolymer confirmed the successful conjugation of FA on PEG-g-PGA copolymer.

Figure SI 8. The UV-Vis spectra and zeta-potential change during the preparation of GNR-PGA or GNR@Pt nanoparticles. (a) The UV-Vis spectra, and (b) Zeta-potential of GNR-PGA nanoparticles prepared at different PGA to Au (PGA/Au) molar ratios; (c) The UV-Vis spectra, and (d) Zeta-potential of GNR@Pt nanoparticles prepared at different cisplatin to Au (Pt/Au) molar ratios.
Figure SI 9. Representative TEM images of CTAB-GNR, GNR-PGA and GNR@Pt nanoparticles (scale bar 50 nm). The black arrows indicated the presence of PGA layer and PEG corona in GNR-PGA or GNR@Pt nanoparticles.

Figure SI 10. Cytotoxicity assay of CTAB-GNR, electrostatically coated GNR@PGA and covalently wrapped GNR-PGA nanoparticles. The cells were incubated the nanoparticles for 24 h and examined by MTT assay.
Figure SI 11. The stability of GNR@Pt in different media. The UV-Vis spectra of GNR@Pt suspension in (a) water, (b) PBS or (c) complete FBS. (d) The representative photographs GNR-PGA or GNR@Pt suspension examined post 1 or 7-day storage in water, PBS or FBS solution.

Figure SI 12. The representative photographs of CTAB-GNR and GNR-PGA nanoparticles in water or PBS solution.
Figure SI 13. The cisplatin release profile of GNR@Pt nanoparticles examined in saline solution.

Figure SI 14. Synthetic route for RhoB-conjugated PEG-g-PGA-SH copolymer (Rho-PEG-g-PGA-SH).
Figure SI 15. $^1$H-NMR spectrum of RhoB-PEG-g-PGA-SH copolymer, PGA homopolymer, RhoB-PEG-NH$_2$ and NH$_2$-PEG-NH$_2$, respectively.
Figure SI 16. Representative TEM images for intracellular localization of GNRs in 4T1 cells after treated with GNR@Pt for 24 h. The enlarged image shown in the right side clearly indicated the lysosome entrapment of GNRs. The black arrow indicated the location of nuclei.
Figure SI 17. H&E staining for major organ collected at the end of animal studies. The mice were intravenously injected GNR-PGA, cisplatin, GNR@Pt or FA-GNR@Pt nanoparticles and selectively treated with NIR laser. From top to bottom: heart (×100), liver (×100), spleen (×100), kidney (×100), and kidney (×200). The black arrows indicated the presence of neutrophil accumulation in heart organ and tubular atrophy in kidney of cisplatin-injected tumor-bearing mice.
Figure SI 18. (a) Laser irradiation induced significant temperature elevation of intratumorally injected 4T1 tumors, and (b) the corresponding infrared photothermal images taken at 60s during laser irradiation.
Figure SI 19. Antitumor efficacy of GNR@Pt nanoparticles administrated through intratumoral injection. (a) Representative whole-body BLI images of 4T1-Luc tumor-bearing mice taken at the 23rd day post first nanoparticle injection; (b) Representative H&E staining micro-photos (× 200) of tumor sections, (c) the tumor growth curves, and (d) tumor mass of mice treated with different modalities.
Figure SI 20. Lung metastasis of primary breast tumors after chemo and photothermal ablation combinational therapy through intratumoral injection; (a) The representative photographs, (b) corresponding BLI images, and (c) corresponding H&E staining micro-photos (× 200) of lung tissues.