Supporting Information for:

Light-Triggered Disassembly of Photo-Responsive Gold Nanovesicles for Controlled Drug Release

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Experimental details:

Materials Tetrachloroauric(III) acid ($HAuCl_4$), sodium borohydride ($NaBH_4$), and (*E*)-cinnamic acid were purchased from China National Medicines Corporation Ltd. Ethanol, tetrahydrofuran (THF), dichloromethane, trimethylamine (Et_3N), and chloroform were purchased from Beijing Chemical Works. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDCI), *N*,*N*-dimethylpyridin-4-amine (DMAP), and poly(vinyl alcohol) (PVA) were purchased from Aladdin. Other reagents were used without further purification. Deionized water (Millipore Milli-Q grade) with resistivity of 18.0 M was used. PS-OH (M_n = 20 000 g/mol) was synthesized by anionic polymerization with n-BuLi as the initiator and epoxyethane as the chain terminator with the molecular weight determined by MALLS-GPC.

Synthesis of α-Truxillic acid. α-Truxillic acid was synthesized according to a literature method (*Macromolecules* 2011, **44**, 159-165) (*E*)-Cinnamic acid was recrystallized by ethanol and water (volume ratio= 1: 1) to give white needle-like crystals. 2.0 g of pure (*E*)-Cinnamic acid crystals was placed on a glass slide and irradiated using a UV lamp (700 mW cm^{-2}, $\lambda = 365$ nm) in air for 18 h. The resulting compound was recrystallized from ethanol to give 1.2 g of white crystals. ^{1}H-NMR: (DMSO-d_6) δ (ppm): 3.81 (2H, β,β'-H), 4.28 (2H, α,α'-H), 7.22–7.33 (10H, aromatic-H).

Synthesis of PS-C4-COOH. EDC.HCl (70 mg, 0.37 mmol) and Et_3N were dissolved in CH_2Cl_2 (5 mL) and the mixture was stirred at room temperature for 30 min. Then, α-truxillic acid was added (109.6 mg, 0.37 mmol). The solution of PS-OH in CH_2Cl_2 (1 mL) was added dropwise to the above solution with stirring. Subsequently, DMAP (5.0 mg, 0.05 mmol) was added slowly. The resulting solution was stirred at room temperature overnight. The esterification process was carried out under the protection of nitrogen. Then, the product was precipitated, filtered, and dried *in vacuo* at 50 ^oC. The structure was verified by ^{1}H NMR.

Synthesis of mPEG-COOH. The reaction of mPEG-OH (2 g) with succinic anhydride was carried in a flask containing 1,4-dioxane and succinic anhydride (0.8 g) in the presence of DMAP (2 mg) as the catalyst at room temperature. The mixture was stirred at room temperature for 24h. Then, the products were precipitated, filtered and dried *in vacuo* at 40 ^{o}C. The structure of the product was verified by NMR.

Synthesis of mPEG-S-S-NH$_2$. Cystamine dihydrochloride was pretreated by adding Et_3N to neutralize the chloride hydride. PEG-COOH (1.2g), EDC.HCl (70 mg, 0.37 mmol), and NHS (42.5 mg, 0.37 mmol) were dissolved in CH_2Cl_2 (5 mL) and stirred at room temperature for 2h. The solution of cystamine in dichloromethane (1 mL) was added dropwise to the solution with stirring under the protection of nitrogen. The resulting solution was stirred at room temperature overnight. Then, the product was precipitated, filtered, and dried *in vacuo* at 50 ^{o}C. The structure of the product was verified by NMR.

Synthesis of PS-C4-PEG. PS-C4-COOH (1.0 g), EDC.HCl (30 mg, 0.37 mmol) and NHS (10 mg, 0.37 mmol) were dissolved in CH_2Cl_2 (5 mL) and stirred at room temperature for 2h. The solution of PEG-S-S-NH$_2$ in dichloromethane (1 mL) was added dropwise to the solution with stirring under the protection of nitrogen. Then, the product was precipitated, filtered, and dried *in vacuo* at 40 ^{o}C and the structure of the product was verified by NMR.

Synthesis of Gold nanoparticles (Au NPs). Au NPs with a spherical shape with diameter of 8 ±1 nm were synthesized using seed-growth method as previously reported (*Langmuir* **2001,** *17*, 6782-6786). Aqueous citrate solution (0.5 mM) was firstly added to the $HAuCl_4$ solution (10 mL, 0.5 mM), and then ice-cold solution of $NaBH_4$ in water (0.6 mL, 0.1 M) was added to the mixture under stirring. The stirring was stopped 1h after the addition of $NaBH_4$ solution, and the resultant seed solution was stored at 30 ^{o}C for 3-5 h. The size of the seed was ~ 3.5 nm. The growth solution was prepared by dissolving $HAuCl_4$ (2.5 mM) and cetyltrimethylammonium bromide (CTAB) (0.08 M) in water. An aqueous solution of ascorbic acid (0.4 mL, 0.1 M) was added to 18 mL growth solution under stirring for 15 min. The resultant solution containing Au NPs was incubated at 30 ^{o}C overnight.

Functionalization of Au NPs with PS-C4-PEG ligand. Chloroform was added to the suspension of as-synthesized Au NPs to remove excess CTAB. Then, the organic layer was centrifuged (13,000 rpm, 20 min, 28 ^{o}C). After centrifugation, the supernatant was removed and the sediment was collected and re-dispersed in 10 mL of 1.5 mg/mL solution of PS-C4- PEG in DMF/THF (1:1). After incubating the mixture for 24 h, the unattached PS-C4-PEG was removed by six cycles of centrifugation (13,000 g, 20 min, 28 °C), each followed by the removal of supernatant and re-dispersed in 10 mL of THF.

Self-assembly of the amphiphilic gold nanoparticles. The self-assembly of BCP-tethered Au NPs was triggered by PVA's selectivity for different blocks. 25 μL of THF suspension of Au@PS/PEG (~2 mg/ml) was added into 200 μL PVA solution (5 mg/mL) and the mixture was gently shaken for mixing. The mixture was kept undisturbed overnight to evaporate residual THF. The supernatant was then removed by centrifugation at 10,000 rpm for 6 min to remove PVA. The centrifugation products were dispersed in water again.

Preparation of amphiphilic gold nanoparticle coated SERS samples.

The GVs suspension with a volume of 50 μL was deposited on the surface of cleaned silicon substrates and dried at room temperature. Then, 20 μL aqueous solution of Rhodamine 6G (R6G, 10^{-7} M) was dropped on the treated substrate. Similarly, the same volume of R6G was dropped on the as-prepared Au NPs treated substrate. To calculate the enhancement factor (EF), the Raman intensity of 0.005 M R6G was com-pared to the signals on a pure Si wafer.

Preparation of the DOX-loaded vesicles and in vitro drug release study. To prepare DOX loaded GVs, DOX was dissolved in 2 mg/mL PVA solution, and then 25 μL THF suspension of BCP-tethered Au NP (~2 mg/mL) was added with gently shaking to form the assemblies. The formed suspension was kept undisturbed overnight to evaporate residual THF. The supernatant was then removed by centrifugation at 10,000 rpm for 6 min and wash with water for 3 times to remove PVA and free DOX. Then, the DOX-loaded GVs were dispersed in 1 mL of pH 7.4 PBS solution for further uses. The release profiles of DOX from the GVs were investigated at 37 °C in pH 7.4 PBS solution. The release of DOX from GVs under UV irradiation was performed for different irradiation time was 10 min after releasing for 20h without light. The amount of DOX released was separated from the vesicles by centrifugation and quantified from the calibration curves of DOX established by the absorbance at 480 nm.

Cellular Studies. Drug release behaviors of the DOX-loaded gold vesicles (GVs) were observed by Confocal Laser Scanning Microscope (CLSM, Olympus FV1200, Tokyo, Japan) toward HeLa cells. HeLa cells were cultured in complete DMEM medium containing 10% (v/v) FBS at 37 °C and 5% (v/v) carbon dioxide. Cells were seeded on sterilized coverslips in 6-well plates at a density of 2.0×10^6 cells per well in 2.0 mL of complete DMEM for 12 h, and then incubated with the GVs at a final DOX concentration of 10.0 mg/L for another 30 min. The cells were treated with or without 10 min of UV irradiation, followed by another incubation for 2h. Thereafter, the cells were fixed with 4% (w/v) paraformaldehyde for 30 min at 25 °C, and counterstained with DAPI (blue) for cell nuclei and Actin-Tracker Green (FITC, green) for cytoskeleton according to the standard protocols provided by the suppliers. CLSM images of the cells were obtained through a CLSM.

In Vitro Cytotoxicity Assay. HeLa cells were seeded in a 96-well plate at a density of 1.0×10^4 cells per well for 12 h and incubated with the DOX, GVs and GVs-DOX for 24h, 48 h and 72h with the concentration of DOX at 2 μg/mL. Thereafter, 10.0 μL of MTT solutions (5.0 g/L) were added into each well and incubated for another 4 h. The medium was carefully discarded, and the resultant formazan crystals were dissolved in 100.0 μL DMSO, and the absorbance at 492 nm was measured by an ELISA microplate reader (Bio-Rad Laboratories, Hercules, CA, U.S.A.). For cytotoxicity measurement under

NIR irradiation, the cells were treated by NIR laser (1.0 W/cm^2, 655 nm) for 40s. For cytotoxicity measurement under UV irradiation, the cells were treated by UV light (254 nm, 100 mW) for 3 min and 10 min. The cell viability was calculated based on the following eqution:

$$Cell\ viability(\%) = \frac{A_{sample}}{A_{control}}$$

Characterization

Gel permeation chromatography coupled to a multi-angle light scattering detector (GPC-MALLS), a combination of light scattering, a refractive index detector (λ = 658 nm), and a viscosity detector. HPLC grade tetrahydrofuran (THF) was used as the mobile phase at 35 ^{o}C (flow rate: 1 mL/min). The data were collected by PL-GPC50 (Keysight Technology). ^{1}H NMR spectra were recorded with a Bruker AV-400 MHz with $CDCl_3$ as the solvent. The UV-vis spectra were obtained on UV-1800 (Shimadzu) UV/Vis System. Fourier-transform infrared (FTIR) spectra were collected on an Equinox 55 spectrophotometer (Bruker). Infrared thermal image was recored using infrared thermal imager (MAG32, Magnity Electronics). The hydrodynamic diameter was measured using a Zetasizer Nano ZS90 (Malvern) instrument with a 50 mW laser of 633 nm at a scattering angle 90^{o}. Scanning Electron Microscope (SEM) image acquired on a Nova NanoSEM 450 with Schottky field emission gun. Raman spectra (irradiated with 5 mW of 532 nm laser light) were taken using HR800-UV spectrometer (Horiba-Jobin Yvon Inc.) coupled with an Olympus metallographic microscope. The spectra were collected using a 100× microscope objective. The integration time was 5s for each spectrum. All the measurements were taken at room temperature. Raman spectra of an aqueous dispersion of the gold assemblies and Rhodamine 6G (R6G, 5ppm in DI water) were measured to determine the enhancement factor (EF) of SERS-encoded vesicles. EF was calculated using the equation EF= $I_{\mathrm{SERS}}N_{\mathrm{normal}}/I_{\mathrm{normal}}N_{\mathrm{SERS}}$, where I_{SERS} and I_{normal} are the peak intensity at 1362 cm^{-1} of MC spectra obtained from the assemblies and the aqueous solution respectively, and N_{SERS} and N_{normal} are the corresponding number of MC molecules in the scattering volume. Confocal laser scanning microscope (CLSM) imaging was performed on FV1200 (Olympus, Tokyo, Japan). Atom force microscope (AFM) was performed on Dimsension Icon with ScanAsyst (Bruker).

Supporting Figures:

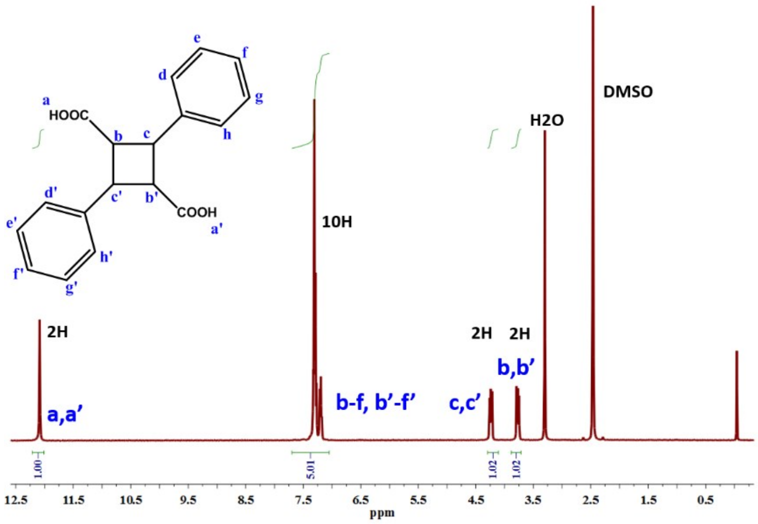

Figure S1. ^{1}H NMR of truxillic acid with d_6-DMSO as the solvent.

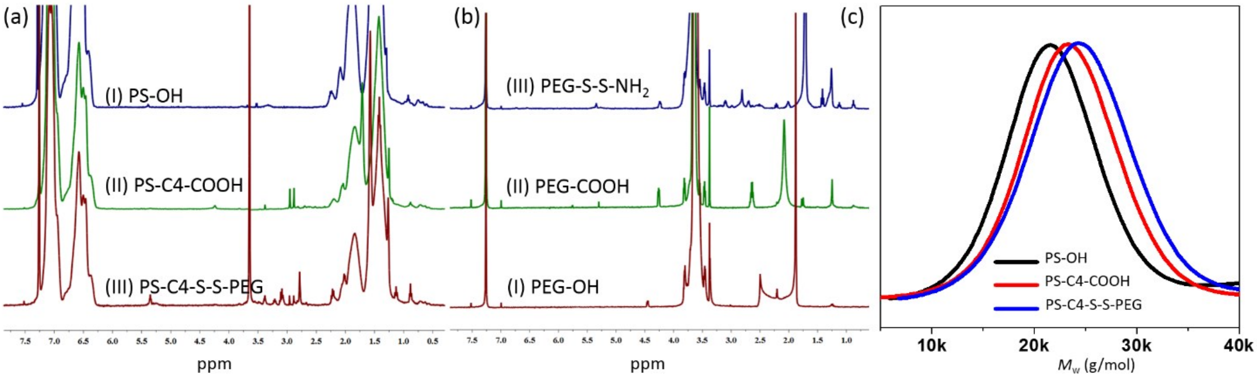

Figure S2. Characterization of the process for PS-C4-PEG synthesis. (a) ^{1}H NMR of the PS-OH, PS-C4-COOH, and PS-C4-PEG; (b) ^{1}H NMR of the mPEG-OH, mPEG-COOH and mPEG-S-S-NH$_2$; (c) Elution curve for PS-OH, PS-C4-COOH and PS-C4-PEG. After the functionality of PS-OH and mPEG-OH, PS-C4-PEG was obtained, and molecular weight of the polymer increased step by step, demonstrating the successful synthesis of the product.

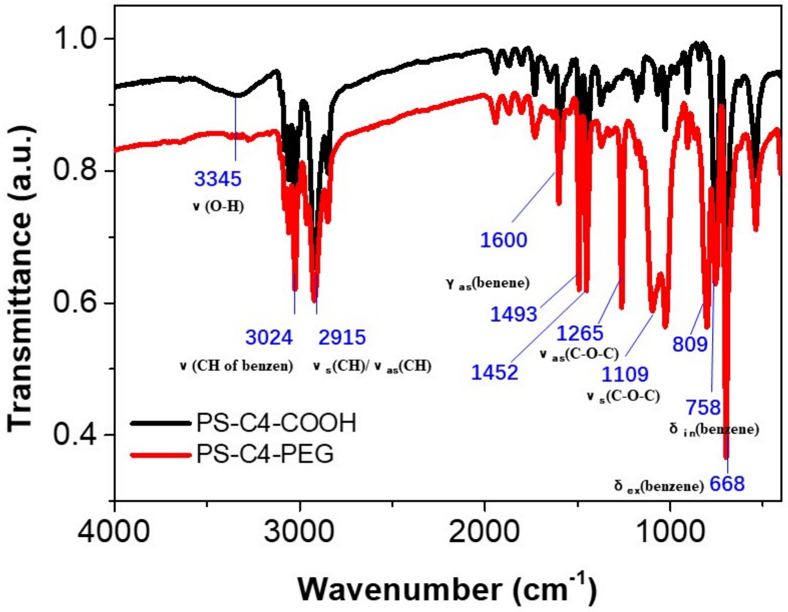

Figure S3 Fourier transformation-infrared spectrum(FT-IR) of PS-C4-COOH and PS-C4-PEG.

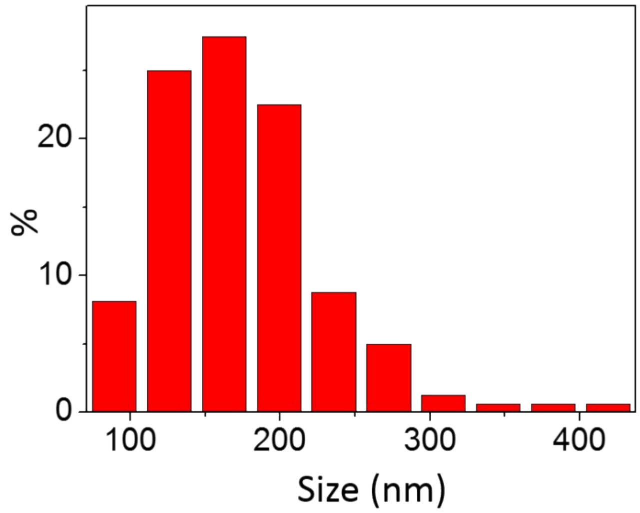

Figure S4. The size distribution of gold nanoparticle vesicles (GVs) statistics from TEM image.

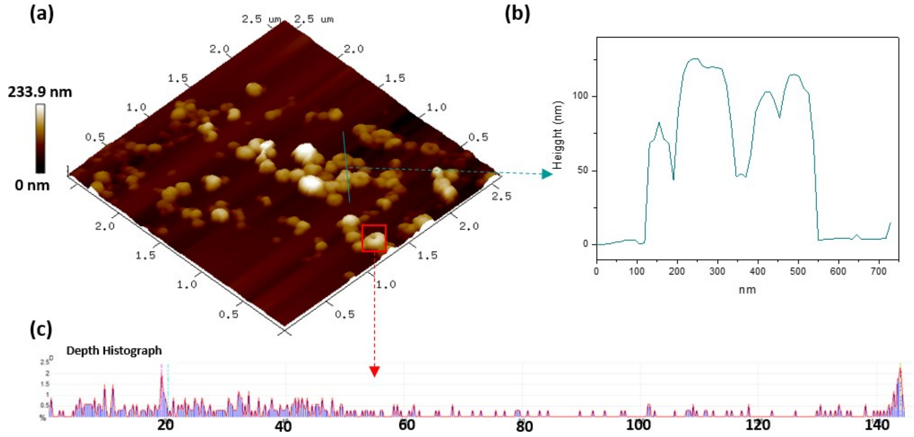

Figure S5. AFM image of GVs: (a) 3D images of GVs, (b) height of the green line colored GVs (c) Depth histography of GVs in the red square.

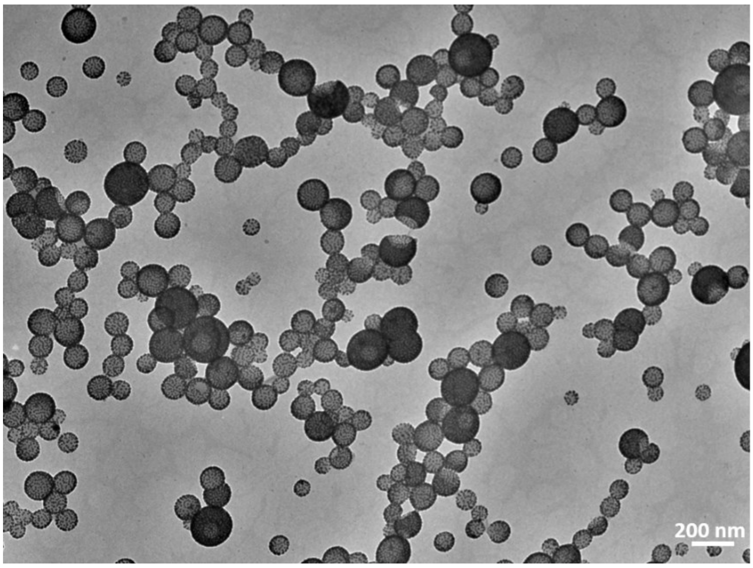

Figure S6. TEM image of GVs after storage for half a year.

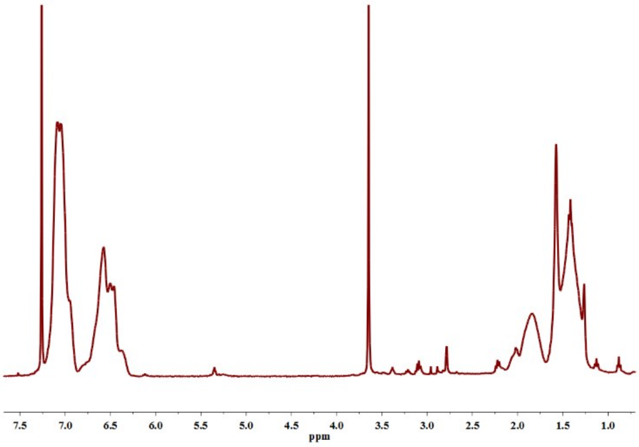

Figure S7. H NMR of the PS-C4-PEG after UV irradiation for 30 min. The appearance at the chemical shift at 6.1 ppm was due to the formation of cinamic acid after dissociation of PS chain.

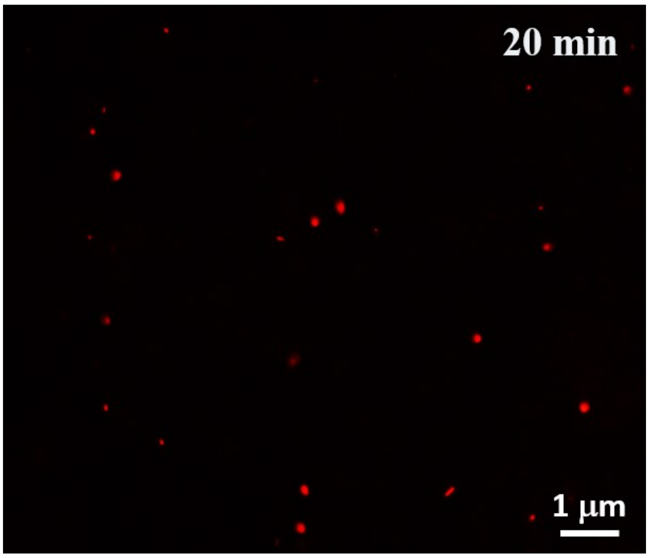

Figure S8 Dark-field fluorescent microscopy image of the GVs showing the scattering color upon the irradiation of UV for 20 min.

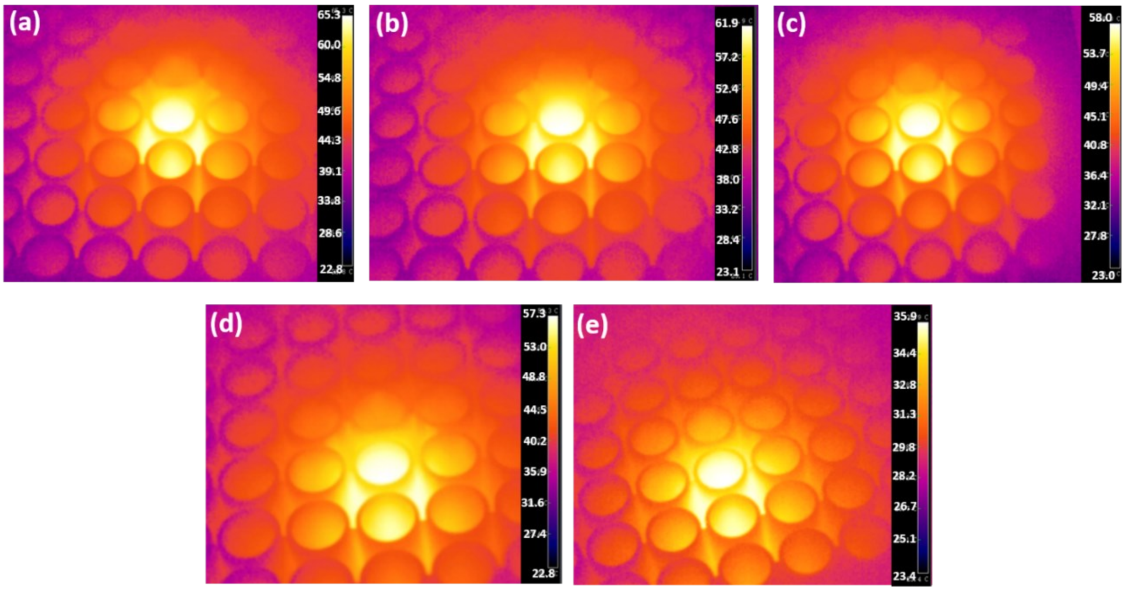

Figure S9 Infrared-thermal image of (a) GVs 40 μg/mL in PBS, (b) GVs 40 μg/mL in PBS after 10 min UV irradiation, (c) GVs 40 μg/mL in PBS after 20 min UV irradiation, (d) GVs 40 μg/mL in PBS after 10 min UV irradiation, (e) PBS solution after 655 nm NIR irradiation for 10 min.

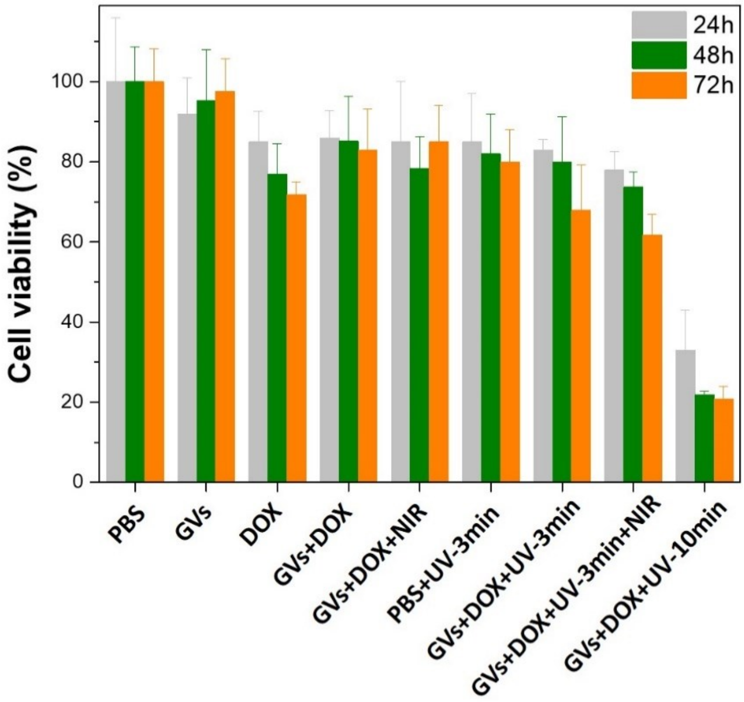

Figure S10. Cell visibility of GVs with various treatment: in the presence and absence of NIR (655 nm NIR 1W for 40 s) irradiation or UV light irradiation for different time (3 min and 10 min).