

Supporting Information

Engineering Cross-Linkable Plasmonic Vesicles for Synergistic Chemo-Photothermal Therapy using Orthogonal Light Irradiation

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Experimental Section

Materials. 2-Isocyanatoethyl methacrylate (stabilized with BHT) was purchased from TCI and used as received. 2-Hydroxyethyl methacrylate (HEMA), 2-bromoethanol, *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC), and paclitaxel (PTX) were purchased from Aldrich. (±)- α -Lipoic acid (98%) was purchased from Alfa Aesar. Borane-*tert*-butylamine complex (TBAB, 95%) was purchased from Acros. Doxorubicin hydrochloride (DOX) was purchased from Iffect Chemphar Co., Ltd. 2-Nitrobenzyl alcohol, dibutyltin dilaurate (DBTL), chloroauric acid (HAuCl₄·4H₂O), sodium citrate, oleylamine (OAm), and tetralin were purchased from Sinopharm Chemical Reagent Co., Ltd. Fetal bovine serum (FBS), penicillin, streptomycin, and Dulbecco's modified Eagle's medium (DMEM) were purchased from GIBCO and used as received. LysoTracker Green and 2-(4-amidinophenyl)-6-indolecarbamide dihydrochloride (DAPI) were purchased from Molecular Probes[®]. 2,2'-Azobisisobutyronitrile (AIBN) was purified by recrystallization from 95% ethanol prior to use. Water was deionized with a Milli-Q SP reagent water system (Millipore) to a specific resistivity of 18.4 M Ω ·cm. Photo-responsive monomer, 2-((((2-nitrobenzyl)oxy)carbonyl)amino) ethyl methacrylate (NBOC),¹ 2-(methacryloyloxy)ethyl 5-(1,2-dithiolan-3-yl)pentanoate (MALA),² 9-diethylamino-2-hydroxy-5H-benzo[*a*]phenoxazin-5-one (NR),³ 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CPADB),⁴ and PEO-based macroRAFT agent⁵ were synthesized according to literature procedures. All other reagents and solvents were purchased from Sinopharm Chemical Reagent Co., Ltd. and used as received unless otherwise noted.

Sample Preparation. Synthetic routes employed for the preparation of Nile red-based monomer (NRMA), diblock copolymers, PNBOC-*b*-PMALA and PEO-*b*-PMALA, are shown in Schemes S1 and S2.

*Synthesis of 9-(diethylamino)-2-(2-hydroxyethoxy)-5H-benzo[*a*]phenoxazin-5-one (NR-OH, Scheme 1).* NR (1 g, 2.99 mmol) and 2-bromoethanol (1.12 g, 8.97 mmol) were dissolved in DMF, and K₂CO₃ (1.24 g, 8.97 mmol) was added. The reaction mixture was maintained at 80 °C for 48 h. After evaporation of the solvent under vacuum, the crude product was purified by column chromatography using dichloromethane/methanol (v/v, 10:1) as the eluent to obtain a purple solid (0.74 g, yield: 65%). ¹H NMR (DMSO-*d*₆, 300 MHz, Figure S1a): 8.03 (m, 1H, ArH), 7.91 (s, 1H, ArH), 7.59 (m, 1H, ArH), 7.25 (m, 1H, ArH), 6.76 (m, 1H, ArH), 6.6 (s, 1H, ArH), 6.15 (s, 1H, ArH), 4.97 (t, 1H, -CH₂-OH), 4.17 (t, 2H, -OCH₂CH₂-), 3.79 (t, 2H, -OCH₂CH₂-), 3.47 (t, 4H, -N(CH₂CH₃)₂), 1.16 (t, 6H, -N(CH₂CH₃)₂).

*Synthesis of 2-(((2-((9-(diethylamino)-5-oxo-5H-benzo[*a*]phenoxazin-2-yl)oxy)ethoxy)carbonyl)amino)ethyl methacrylate (NRMA, Scheme S1).* NR-OH (500 mg, 1.32 mmol) and 2-

isocyanatoethyl methacrylate (226 mg, 1.45 mmol) were dissolved in 30 mL of dry dichloromethane, to which 50 μ L of DBTL was added. The reaction mixture was maintained at 40 °C for 12 h. After the reaction was completed, the reaction solution was washed with saturated brine, dried over MgSO₄, and then the solvent was removed under reduced pressure. After further purification by flash column chromatography on alkaline aluminum oxide using ethyl acetate as an eluent, the solvent was removed under reduced pressure. The named product was dried overnight in a vacuum oven at room temperature, affording NRMA monomer as a purple solid (528 mg, yield: 75%). ¹H NMR (DMSO-*d*₆, δ , 300 MHz, Figure S1b): 8.03 (m, 1H, ArH), 7.91 (s, 1H, ArH), 7.60 (m, 1H, ArH), 7.23 (m, 1H, ArH), 6.80 (m, 1H, ArH), 6.64 (s, 1H, ArH), 6.19 (s, 1H, ArH), 6.05 (s, 1H, -C(CH₃)CHH), 5.65 (broad, 2H, -C(CH₃)CHH, -NH-COO-), 4.36 (s, 4H, -COO-CH₂-, -NHCOO-CH₂-), 4.08 (m, 2H, ArO-CH₂-), 3.49 (t, 4H, -N(CH₂CH₃)₂), 3.29 (t, 2H, -OCONH-CH₂-), 1.85 (s, 3H, -C(CH₃)-), 1.17 (t, 6H, -N(CH₂CH₃)₂). ¹³C NMR (DMSO-*d*₆, 75 MHz, Figure S2a): 181.80, 166.96, 161.23, 156.72, 152.19, 151.30, 146.92, 138.58, 136.23, 134.02, 131.41, 127.73, 126.38, 125.55, 124.40, 118.38, 110.55, 106.60, 104.51, 96.42, 67.21, 63.74, 44.91, 18.41, 12.92. ESI-MS (m/z): [M+H]⁺ calcd. for C₂₉H₃₂N₃O₇, 534.224; found, 534.223 (Figure S2b).

Synthesis of PNBOC Homopolymers (Scheme S1). Typically, CPADB (50 mg, 0.16 mmol), NBOC (1.5 g, 4.72 mmol), AIBN (5 mg, 0.03 mmol), and 1,4-dioxane (4.8 mL) were charged into a sealed tube flask equipped with a magnetic stirring bar. The flask was carefully degassed by three freeze-pump-thaw cycles and sealed under vacuum. After thermostating at 70 °C for 24 h, the reaction flask was quenched into liquid nitrogen, opened, and diluted with 1,4-dioxane; the reaction mixture was precipitated into an excess of diethyl ether. The above dissolution-precipitation cycle was repeated twice. After drying in a vacuum oven, PNBOC₂₇ (**P1**) was obtained as a reddish solid (1.3 g, yield: 87%). GPC analysis revealed an *M_n* of 9.4 kDa and an *M_w/M_n* of 1.18 (Table S1). The degree of polymerization, DP, of PNBOC was determined to be 27 by ¹H NMR analysis (Table S1, Figure S3a). According to a similar procedure, PNBOC₅₀ (**P2**) was also synthesized (Table S1).

*Synthesis of PNBOC-*b*-PMALA Diblock Copolymers (Scheme S2).* Typical procedures employed for the RAFT synthesis of PNBOC₂₇-*b*-PMALA₇ (**BP1**) are as follows. PNBOC₂₇ (**P1**, 400 mg, 0.048 mmol), MALA (153 mg, 0.48 mmol), AIBN (1.6 mg, 0.01 mmol), and 1,4-dioxane (1 mL) were charged into a sealed tube equipped with a magnetic stirring bar. The flask was carefully degassed by three freeze-pump-thaw cycles and sealed under vacuum. After thermostating at 60 °C for 24 h, the reaction flask was quenched into liquid nitrogen, opened, and diluted with 1,4-dioxane; the reaction mixture was precipitated into an excess of diethyl

ether. The above dissolution-precipitation cycle was repeated twice. After drying in a vacuum oven, PNBOC₂₇-*b*-PMALA₇ (**BP1**) was obtained as a reddish solid (400 mg, yield: 72 %). GPC analysis revealed an M_n of 12.5 kDa and an M_w/M_n of 1.22 (Table S1, Figure S3b). The degree of polymerization, DP, of PMALA was determined to be 7 by ¹H NMR analysis. According to a similar procedure, PNBOC₅₀-*b*-PMALA₅ (**BP2**) was also synthesized (Table S1). NRMA-labeled diblock copolymer, P(NBOC_{0.98}-*co*-NRMA_{0.02})₂₇-*b*-PMALA₇ (**BP3**), was synthesized using P(NBOC-*co*-NRMA) as the macroRAFT agent (Scheme S2).

Synthesis of PEO-b-PMALA Diblock Copolymers (Scheme S2). Typical procedures employed for the RAFT synthesis of PEO₄₅-*b*-PMALA₅ are as follows. PEO₄₅-CTA (500 mg, 0.22 mmol), MALA (700 mg, 2.2 mmol), AIBN (4 mg, 0.024 mmol), and 1,4-dioxane (4 mL) were charged into a sealed tube equipped with a magnetic stirring bar. The tube was carefully degassed by three freeze-pump-thaw cycles and sealed under vacuum. After thermostating at 60 °C for 24 h, the reaction flask was quenched into liquid nitrogen, opened, and diluted with 1,4-dioxane; the reaction mixture was precipitated into an excess of diethyl ether. The above dissolution-precipitation cycle was repeated twice. After drying in a vacuum oven, PEO₄₅-*b*-PMALA₅ (**BP4**) was obtained as a red solid (500 mg, yield: 42%). GPC analysis revealed an M_n of 4.7 kDa and an M_w/M_n of 1.21 (Table S1, Figure S3c). The degree of polymerization, DP, of PMALA block, was determined to be 5 by ¹H NMR analysis. According to a similar procedure, PEO₁₁₃-*b*-PMALA₅ (**BP5**) was also synthesized (Table S1, Figure S3d).

Synthesis of Gold Nanoparticles (AuNPs). Hydrophobic oleylamine (OAm)-coated AuNPs with an average diameter of 3.0 nm⁶ and sodium citrate-stabilized AuNPs with 13.0 nm in diameter⁷ were prepared according to previously reported procedures.

Typically, for the preparation of OAm-stabilized Au NPs, tetralin solution (10 mL) of OAm (10 mL) and HAuCl₄·4H₂O (0.1 g) was prepared at 25 °C and magnetically stirred under N₂ flow for 30 min. A reducing solution of TBAB (0.5 M) in a mixture of tetralin (1 mL) and OAm (1 mL) was then injected. The mixture was allowed to react at 25 °C for 2 h before methanol (60 mL) was added to precipitate the Au NPs. Au NPs were harvested by centrifugation (3,000 rpm, 2 min), washed with methanol and re-dispersed in cyclohexane with a concentration of 20 mg/mL. The resulting OAm-stabilized AuNPs were transformed to CTAB-stabilized AuNPs using a ligand exchange protocol.⁸

For the preparation of sodium citrate-stabilized AuNPs, into a boiling aqueous solution of HAuCl₄ (30 mg, 200 mL) under vigorous stirring, an aqueous solution (2 mL) of sodium citrate (102 mg) was quickly injected. After reaction at 100 °C for 30 min, the solution was cooled down to room temperature. The resulting Au colloidal solution was employed for subsequent

surface modification without further purification.

Preparation of Amphiphilic AuNPs via Ligand Exchange Processes. For the surface modification of OAm-stabilized AuNPs (~3 nm in diameter), 0.5 mL of AuNPs in cyclohexane was diluted with 10 mL of chloroform under magnetically stirring. Then, a chloroform solution (4 mL) comprising PNBOC₂₇-*b*-PMALA₇ (**BP1**, 50 mg) and PEO₁₁₃-*b*-PMALA₅ (**BP5**, 46 mg) was added and stirred overnight at room temperature. After the solvent was removed by rotary evaporation, **BP1**- and **BP5**-functionalized AuNPs (abbreviated as Au₃@PEO₁₁₃/PNBOC₂₇, **PV-1**) were re-dispersed in THF, followed by centrifugation in a mixture of THF/ethanol (1:1, v/v) six times to remove free **BP1** and **BP5**. The purified **PV-1** vesicles were re-dispersed in THF for further use.

For the surface modification of the sodium citrate-stabilized AuNPs (~13 nm in diameter), PNBOC₅₀-*b*-PMALA₅ (**BP2**, 20 mg) and PEO₄₅-*b*-PMALA₅ (**BP4**, 5 mg) were dissolved in DMF/THF (80 mL, 1:1, v/v). Then, an aqueous dispersion of AuNPs (2 mg/mL) was slowly added to the mixture under sonication for 30 min. The resulting solution was stirred overnight at room temperature. **BP2**- and **BP4**-functionalized AuNPs (abbreviated as Au₁₃@PEO₄₅/PNBOC₅₀, **PV-2**) were purified by centrifugation three times and re-dispersed in THF for further use.

Self-Assembly of Amphiphilic AuNPs. To a THF solution of **PV-1** or **PV-2** (1 mL, 2 mg/mL), 4 mL of deionized water was injected by a syringe pump at a rate of 6 mL/h under gentle stirring. After water addition, the solution was subjected to dialysis against deionized water to remove the organic solvent. In addition, flash precipitation was employed to self-assemble hybrid **PV-1** amphiphiles. Briefly, 1 mL of THF dispersions (2.0 mg/mL) of hybrid AuNPs amphiphiles was injected into 4 mL of deionized water in one shot under vigorous magnetic stirring (1500 rpm), followed by dialysis against deionized water to remove the organic solvents.

Preparation of DOX/PTX Co-Loaded PV-2 Vesicles. Typical procedures employed for the encapsulation of hydrophilic doxorubicin hydrochloride (DOX) and hydrophobic paclitaxel (PTX) into the hybrid **PV-2** vesicles are as follows. 1 mg of PTX was dissolved in the THF dispersion of **PV-2**. Subsequently, 2 mg of DOX in deionized water (4 mL) was then added into the **PV-2** dispersion via a syringe pump at a rate of 6 mL/h under gentle stirring, followed by dialysis against deionized water to remove un-loaded drugs and the organic solvent. The drug loading content (DLC) of DOX was determined to be 7.5 wt% by measuring the fluorescence at 590 under excitation at 480 nm while the DLC of PTX was calculated to 6.8 wt% by HPLC, respectively.

Light-Irradiation Triggered Release of DOX and PTX from PV-2 Vesicles. To study light irradiation-induced co-release of PTX and DOX from the **PV-2** vesicles. 0.5 mL of **PV-2** vesicles dispersion was placed in a dialysis tube (MWCO: 3,500 Da). After irradiation with a 410 nm LED lamp under varying durations, the dialysis tube was placed in 10 mL of PBS media at 37 °C. Periodically, 10 mL of external buffer solution was removed and replaced with equal volume of fresh PBS buffer. Upon each sampling, 10 mL of buffer solution was lyophilized. The DOX and PTX concentrations were quantified by fluorescence and HPLC measurements against established calibration curves, respectively.

In vitro Drug Release from PV-2 Vesicles Observed with Confocal Laser Scanning Microscopy (CLSM). HepG2 cells were plated onto glass-bottom Petri dishes at a density of 80,000 cells per dish, then cultured in DMEM supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL), and streptomycin (100 µg/mL) for 24 h at 37 °C in CO₂-air (5:95). Then DOX/PTX co-loaded hybrid **PV-2** vesicles were added at a final DOX-equivalent concentration of 5 µg/mL. After incubation for two hours, the cells were then washed with PBS, and 180 µL of fresh medium was added. After irradiated with a 410 nm LED lamp for 10 min, the cells were then incubated for another 2 h, 6 h, and 10 h, respectively. Cells were rinsed with PBS buffer three times. Afterward, cell nuclei were stained with DAPI and the endosomes and lysosomes were stained with LysoTracker Green, respectively. The images were then taken using a confocal laser scanning microscopy. The red fluorescence of DOX was excited using a 543 nm laser with the emission channel was set to be 570-650 nm. DAPI and LysoTracker green were excited by 405 nm and 488 nm lasers and the emission channels were set to be 420-480 nm and 500-550 nm, respectively. Cells after incubation with hybrid **PV-2** vesicles without 410 nm LED light irradiation was used as the control group.

In situ Observation of the Morphology Change of HepG2 Cells with Cross-Linked PV-2 Vesicles under Photo-Irradiation. HepG2 cells were plated onto glass-bottom Petri dishes at a density of 80,000 cells per dish, then cultured in DMEM supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL), and streptomycin (100 µg/mL) for 24 h at 37 °C in CO₂-air (5:95). Then hybrid **PV-2** vesicles were added at a final concentration of 20 µg/mL. After incubation for 2 h, the cells were washed with PBS buffer three times, and then 180 µL of fresh culture medium was added. The cells were further cultured in an online incubator. The cells were *in situ* irradiated with a 543 nm laser (~0.8 W/cm²) for varying times and were observed by CLSM. The cells without adding hybrid **PV-2** vesicles were used as the control.

In Vitro Cytotoxicity Assay. HepG2 cells were plated onto glass-bottom Petri dishes at a density of 80,000 cells per dish, then cultured in DMEM supplemented with 10% fetal bovine

serum (FBS), penicillin (100 units/mL), and streptomycin (100 µg/mL) for 24 h at 37 °C in CO₂-air (5:95). Then, drug-free **PV-2** vesicles were added at different concentrations for 2 h. The cells were washed with PBS buffer and 180 µL of fresh culture medium was added. After that, the cells were or were not subjected to 410 nm light irradiation for 10 min. After further incubation for another 48 h, the cell viability was measured using the MTT assay.

Assessment of the Chemotherapy and Photothermal Therapy (PTT) of Hybrid PV-2 Vesicles. DOX/PTX co-loaded or empty **PV-2** vesicles (266 µg/mL) were added and incubated with HepG2 cells for 2 h. Afterward, the cells were washed with PBS, and 180 µL of fresh medium was added. The cells were treated with or without 410 nm light irradiation for 10 min and subsequent with or without 560 nm light irradiation for 30 min. After further incubation for another 48 h, the cell viability was measured using the MTT assay.

Live/Dead Assay of HepG2 Cells. The synergistic treatment of chemotherapy and PTT was further assessed by a live/dead assay. Briefly, drug-free or DOX/PTX co-loaded **PV-2** vesicles was added to HepG2 cells and was further incubated for 2 h. Afterward, the cells were washed with PBS and 180 µL of fresh medium was added. The cells were treated with or without 410 nm light irradiation for 10 min and subsequent with or without 560 nm light irradiation for 30 min. After further incubation for another 48 h, the cells were stained with fluorescein diacetate and propidium iodide to visualize the populations of live and dead cells, respectively. The cells were imaged by CLSM using 488 nm and 543 nm lasers and the emission channels were set at 500–530 nm and 560-700 nm, respectively.

*Calculation the Grafting Numbers of PNBOC-*b*-PMALA and PEO-*b*-PMALA Chains on AuNPs.* The weight of a single AuNP was calculated using the following equation:

$$m_{\text{Au}} = \frac{4\pi\rho r_{\text{Au}}^3}{3} \quad (1)$$

Where m_{Au} is the weight of individual AuNP; π is 3.14159; ρ is the density of gold ($19.3 \times 10^3 \text{ Kg/m}^3$); r_{Au} is the average radius of AuNPs.

It was hypothesized that all the original small molecule ligands (i.e., CTAB and sodium citrate) were exchanged during the ligand exchange procedure. The residual weights of hybrid AuNPs could be calculated from the following equation:

$$W_{\text{retention}\%} = \frac{W_{\text{AuNP}}}{W_{\text{AuNP}} + W_{\text{PNBOC}} + W_{\text{PEG}}} \quad (2)$$

Where $W_{\text{retention}\%}$ is the weight retention of hybrid AuNP@PEG/PNBOC amphiphiles determined by TGA analysis; W_{AuNP} is the weight of AuNP as calculated from equation (1); W_{PNBOC} is the weight of grafted PNBOC-*b*-PMALA chains; W_{PEG} is the weight of grafted PEG-*b*-PMALA chains.

W_{PEG} and W_{PNBOC} can be calculated from the following equations:

$$W_{\text{PEG}} = \frac{N_{\text{PEG}}}{N_{\text{A}}} \times M_{\text{PEG}} \quad (3)$$

$$W_{\text{PNBOC}} = \frac{N_{\text{PNBOC}}}{N_{\text{A}}} \times M_{\text{PNBOC}} \quad (4)$$

Where N_{PEG} and N_{PNBOC} are the numbers of grafted PEG-*b*-PMALA and PNBOC-*b*-PMALA chains on the AuNPs, respectively; M_{PEG} and M_{PNBOC} are the molecular weights of PEG-*b*-PMALA and PNBOC-*b*-PMALA chains, respectively; N_{A} is the Avogadro's Constant ($6.0221415 \times 10^{23} \text{ mol}^{-1}$).

The ratios ($N_{\text{PEG}}/N_{\text{PNBOC}}$) of PEG-*b*-PMALA and PNBOC-*b*-PMALA chains on the AuNPs were determined by ^1H NMR results (Figure S7a,c). Therefore, the grafting numbers of **BP1** (PNBOC₂₇-*b*-PMALA₇) and **BP5** (PEO₁₁₃-*b*-PMALA₅) chains on **PV-1** were calculated to be 18 and 7 and the grafting numbers of **BP2** (PNBOC₅₀-*b*-PMALA₅) and **BP4** (PEO₄₅-*b*-PMALA₅) chains on **PV-2** were determined to be 148 and 100, respectively.

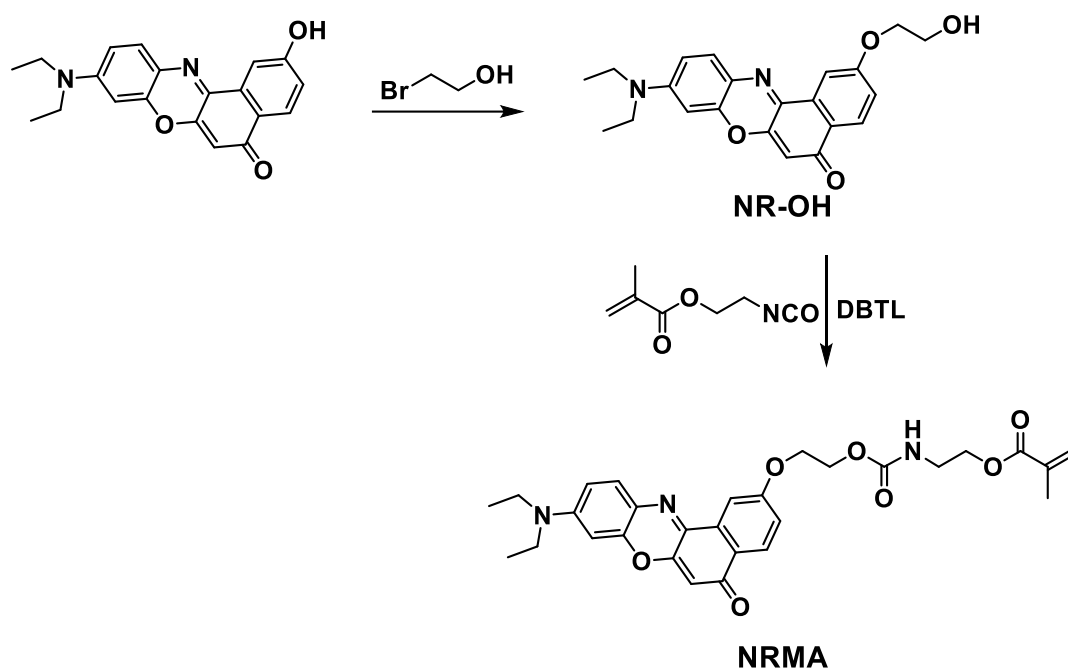
Characterization.

All nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AV300 NMR 300 MHz spectrometer operated in the Fourier transform mode. Deuterated chloroform (CDCl_3) or dimethyl sulfone ($\text{DMSO-}d_6$) were used as the solvents. Molecular weights and molecular weight distributions were determined by gel permeation chromatography (GPC) equipped with Waters 1515 pump and Waters 2414 differential refractive index detector (set at 30 °C). It used a series of two linear Styragel columns (HR2 and HR4) at an oven temperature of 45 °C. The eluent was THF at a flow rate of 1.0 mL/min. A series of low polydispersity polystyrene standards were employed for calibration. Fourier transform infrared (FT-IR) spectra were recorded on a Bruker VECTOR-22 IR spectrometer. The spectra were collected over 64 scans with a spectral resolution of 4 cm^{-1} . UV/vis absorbance spectra were conducted on a TU-1910 double-beam UV-vis spectrophotometer (Puxi General Instrumental Company, China). XPS was performed using an ESCALAB-MK II instrument (VG, UK) equipped with a monochromatic Al $K\alpha$ X-ray source. Fluorescence experiments were conducted on an F-4600 (Hitachi) spectrofluorometer. Dynamic laser light scattering (LLS) measurements were conducted on a commercial spectrometer (ALV/DLS/SLS-5022F) equipped with a multi-tau digital time correlator (ALV5000) and a cylindrical 22 mW UNIPHASE He-Ne laser ($\lambda_0 = 632 \text{ nm}$) as the light source. Scattered light was collected at a fixed angle of 173° for the duration of ~5 min. Distribution averages and particle size distributions were computed using cumulants

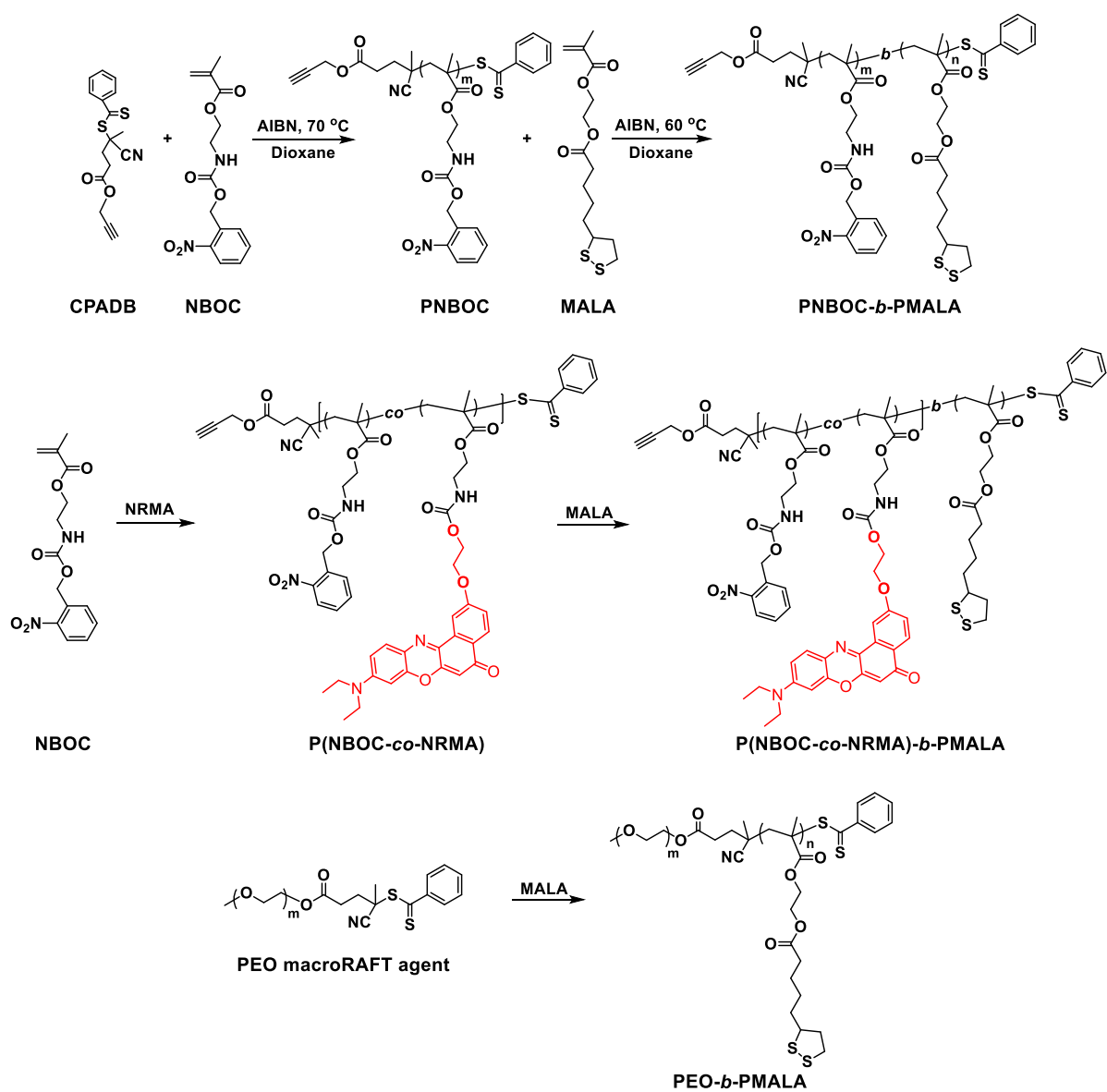
analysis and CONTIN routines. All data were averaged over three consecutive measurements. Inductively coupled plasma atomic emission spectrometry (ICP–AES) (Perkin Elmer Corporation Optima 7300 DV) was used for Au (III) content analysis. Thermogravimetric analysis (TGA) was performed in air using a Perkin Elmer Diamond TG/DTA instrument at a heating rate of 10 °C/min. Transmission electron microscopy (TEM) observations were conducted on a Hitachi H-800 electron microscope at an acceleration voltage of 200 kV. The samples for TEM observations were prepared by placing 10 μ L of aqueous dispersions (0.2 g/L) of assembled amphiphilic AuNPs on copper grids coated with thin films of Formvar and carbon successively. The samples for SEM observations were prepared according to the similar procedures used for TEM. Confocal laser scanning microscopy (CLSM) images were acquired on a Leica TCS SP5 microscope. Computed tomography (CT) scanning was conducted on a clinical CT scanner (Discovery CT 750 HD, GE). The commercially available CT contrast agent, Xenetix[®] 300, was used as the control. The aqueous dispersions of **PV-1** vesicles and Xenetix[®] 300 in centrifugation tubes at varying concentrations were scanned with the following parameters: tube voltage: 120 keV, tube current: 40 mA. The X-ray attenuation values in Hounsfield units (HU) were calculated by the mean counts of the regions of interest (ROI).

Reference

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Scheme S1. Synthetic routes employed for the preparation of NRMA monomer.



Scheme S2. Synthetic routes employed for the preparation of diblock copolymers, PNBOC-*b*-PMALA, P(NBOC-*co*-NRMA)-*b*-PMALA, and PEO-*b*-PMALA, respectively.

Table S1: Molecular Parameters of Homopolymers and Diblock Copolymers Used in This Study.

Entry	Samples ^a	<i>M_n</i> (kDa) ^a	<i>M_n</i> (kDa) ^b	<i>M_w</i>/<i>M_n</i> ^b
P1	PNBOC ₂₇	8.3	9.4	1.18
P2	PNBOC ₅₀	15.9	23.1	1.25
BP1	PNBOC ₂₇ - <i>b</i> -PMALA ₇	10.5	12.5	1.22
BP2	PNBOC ₅₀ - <i>b</i> -PMALA ₅	17.5	25.4	1.33
BP3	P(NBOC _{0.98-co} -NRMA _{0.02}) ₂₇ - <i>b</i> -PMALA ₇	10.7	13.2	1.28
BP4	PEO ₄₅ - <i>b</i> -PMALA ₅	3.6	4.7	1.21
BP5	PEO ₁₁₃ - <i>b</i> -PMALA ₅	6.6	7.8	1.28

^a Calculated from ¹H NMR results. ^b Determined by GPC using THF as the eluent (1.0 mL/min).

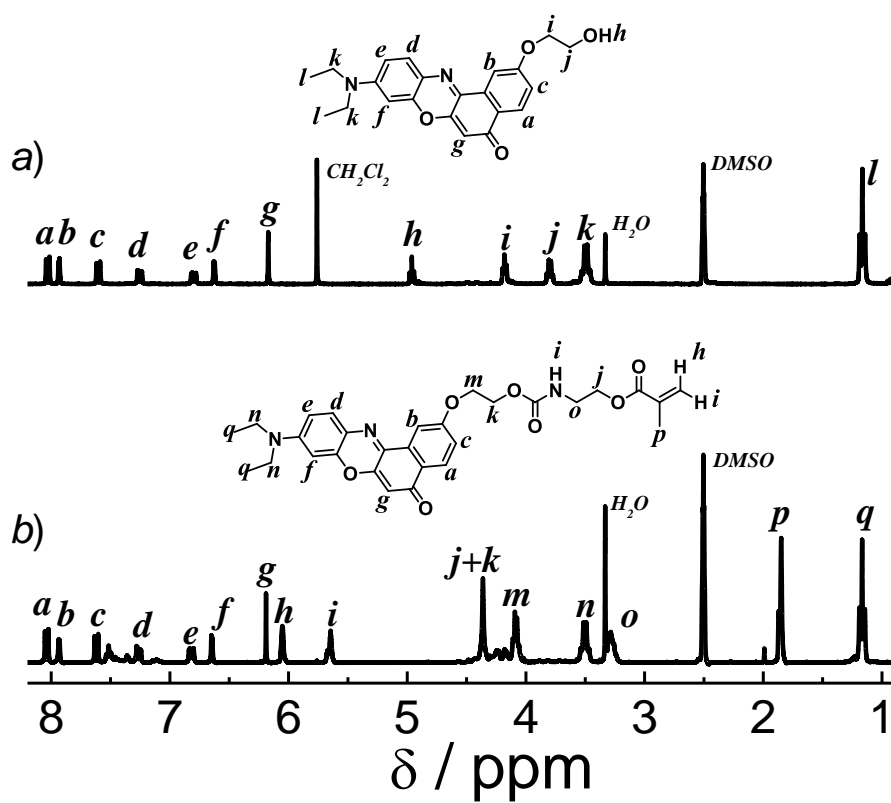


Figure S1. ^1H NMR spectra recorded in $\text{DMSO-}d_6$ for (a) NR-OH precursor and (b) NRMA monomer.

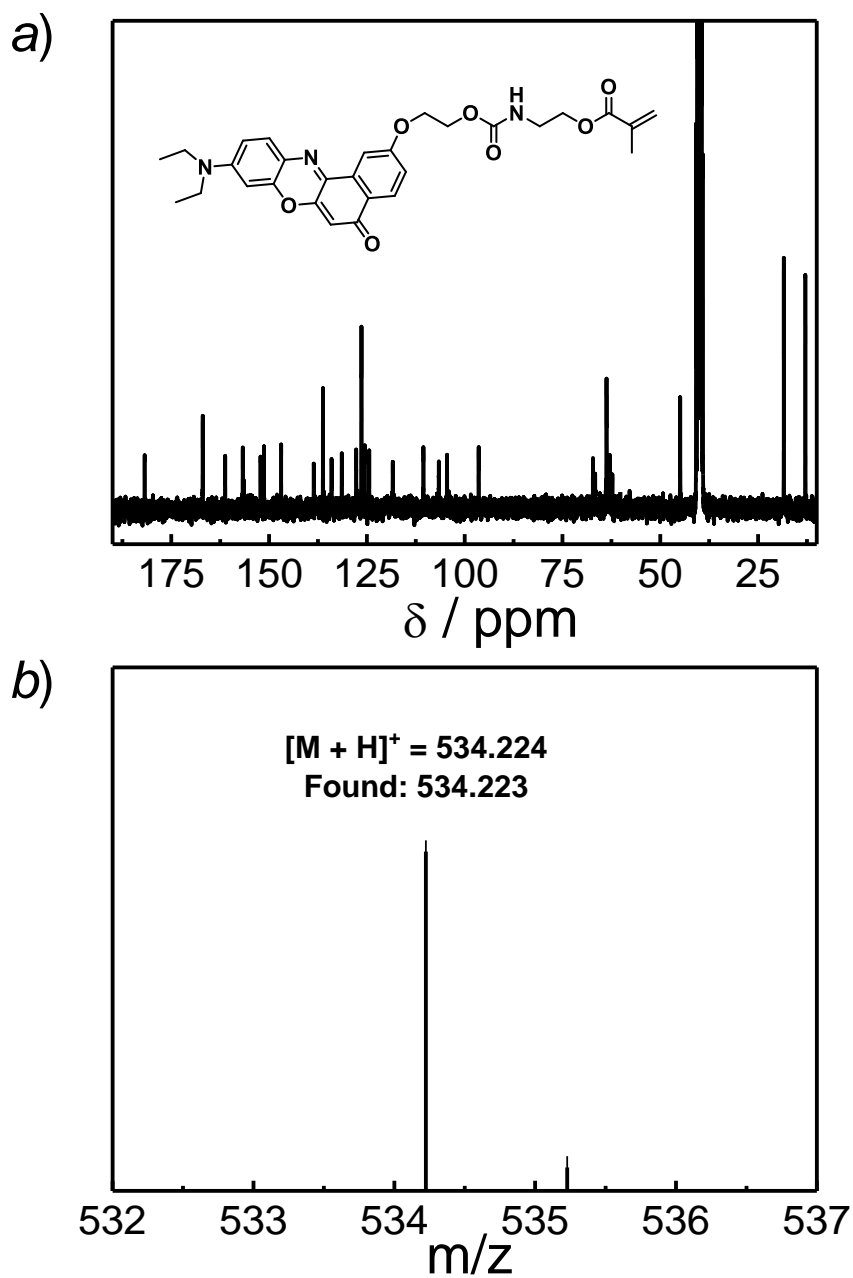


Figure S2. (a) ^{13}C NMR spectrum in $\text{DMSO-}d_6$ and (b) ESI-MS spectrum recorded for NRMA monomer.

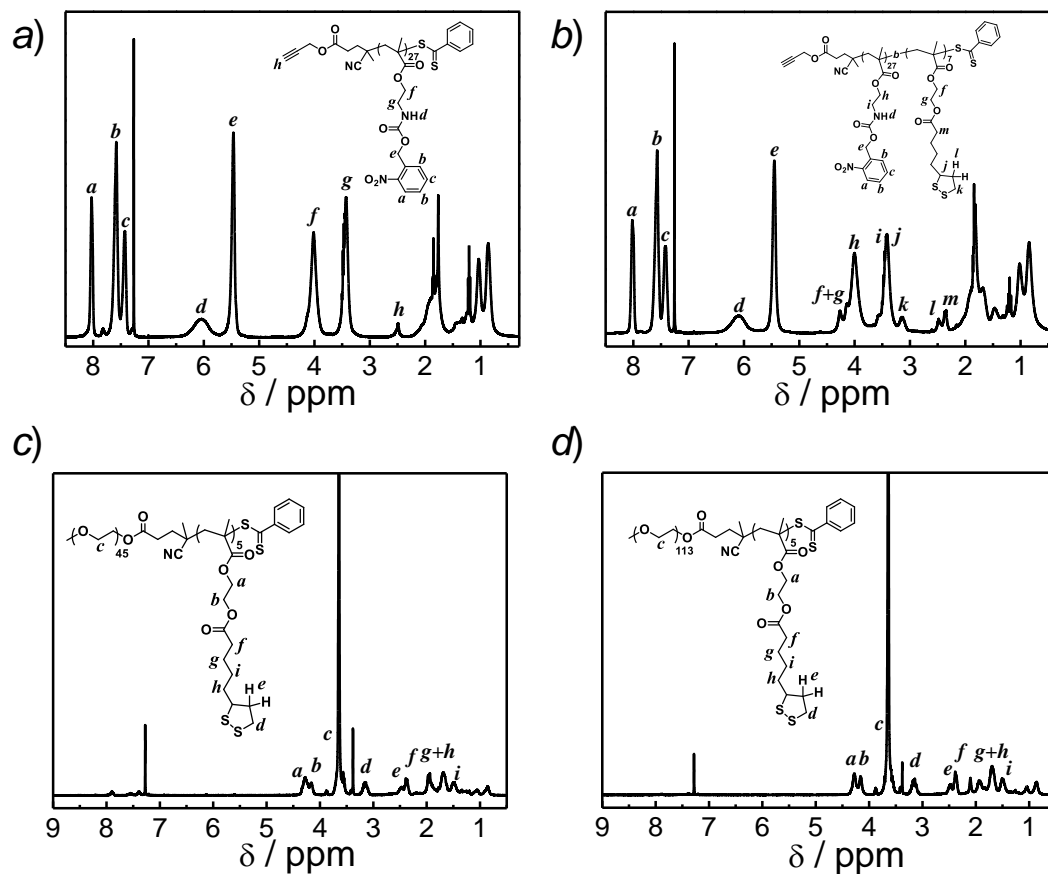


Figure S3. ^1H NMR spectra recorded in CDCl_3 for (a) PNBOC₂₇ precursor (**P1**), (b) PNBOC₂₇-b-PMALA₇ (**BP1**), (c) PEO₄₅-b-PMALA₅ (**BP4**), and (d) PEO₁₁₃-b-PMALA₅ (**BP5**), respectively.

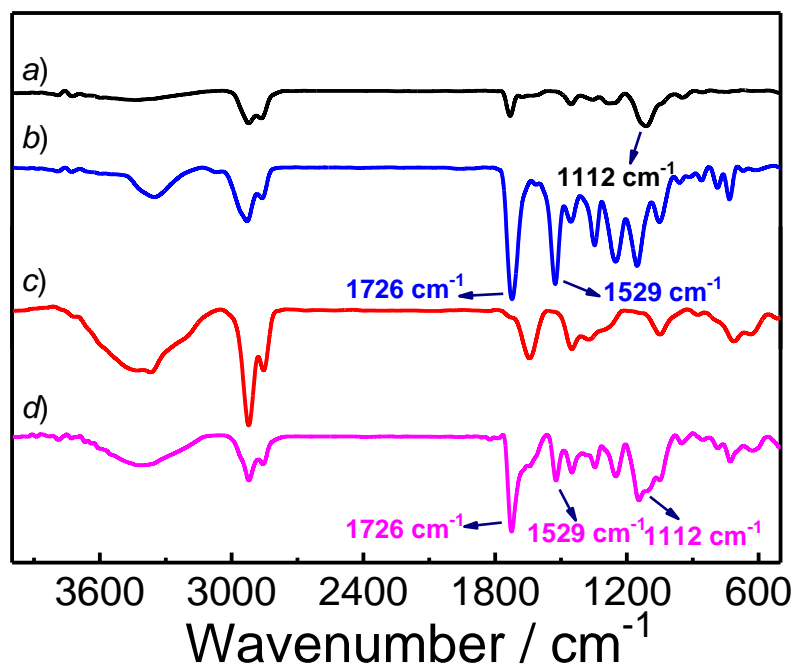


Figure S4. FT-IR spectra recorded for (a) PEO₁₁₃-*b*-PMALA₅ (**BP5**), (b) PNBOC₂₇-*b*-PMALA₇ (**BP1**), (c) OAm-stabilized AuNPs, and (d) hybrid **PV-1** amphiphiles.

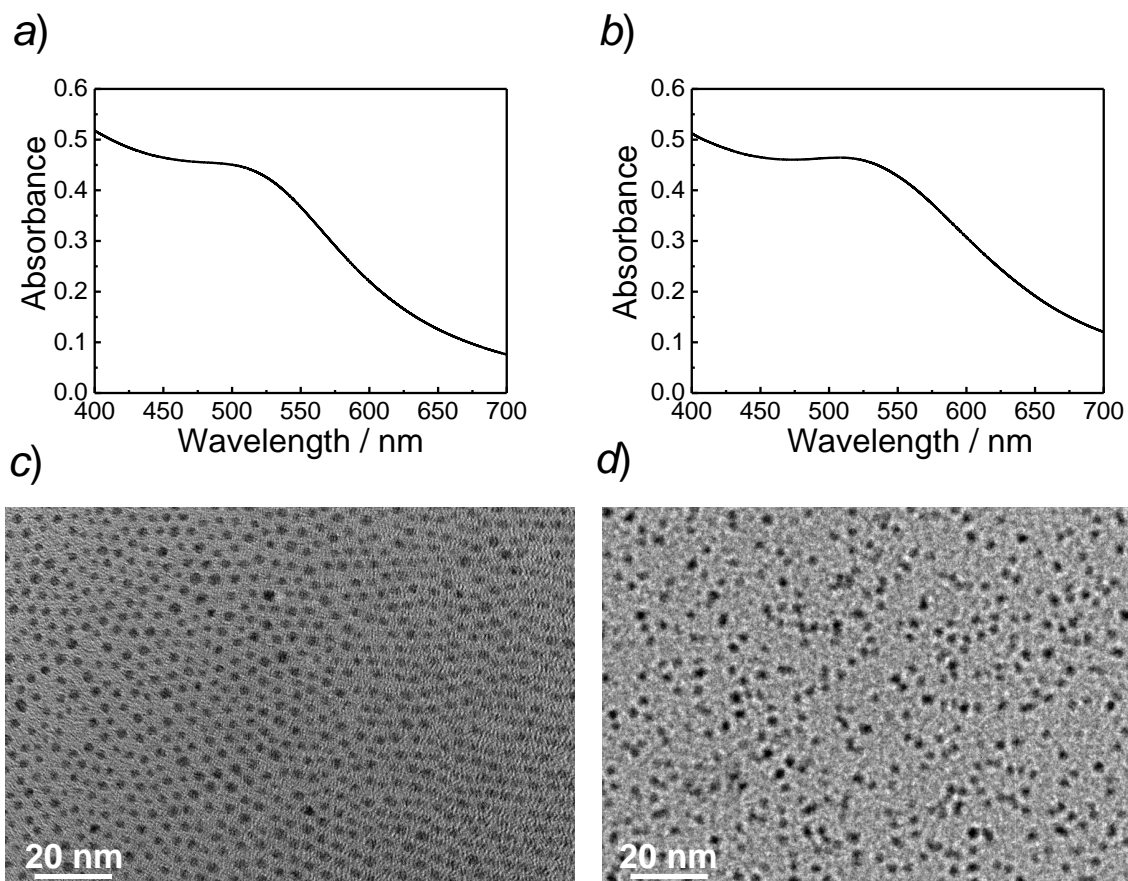


Figure S5. (a,b) UV/vis absorbance spectra and (c,d) TEM images of (a,c) OAm-stabilized AuNPs (3 nm) in cyclohexane and (b,d) hybrid **PV-1** amphiphiles in THF.

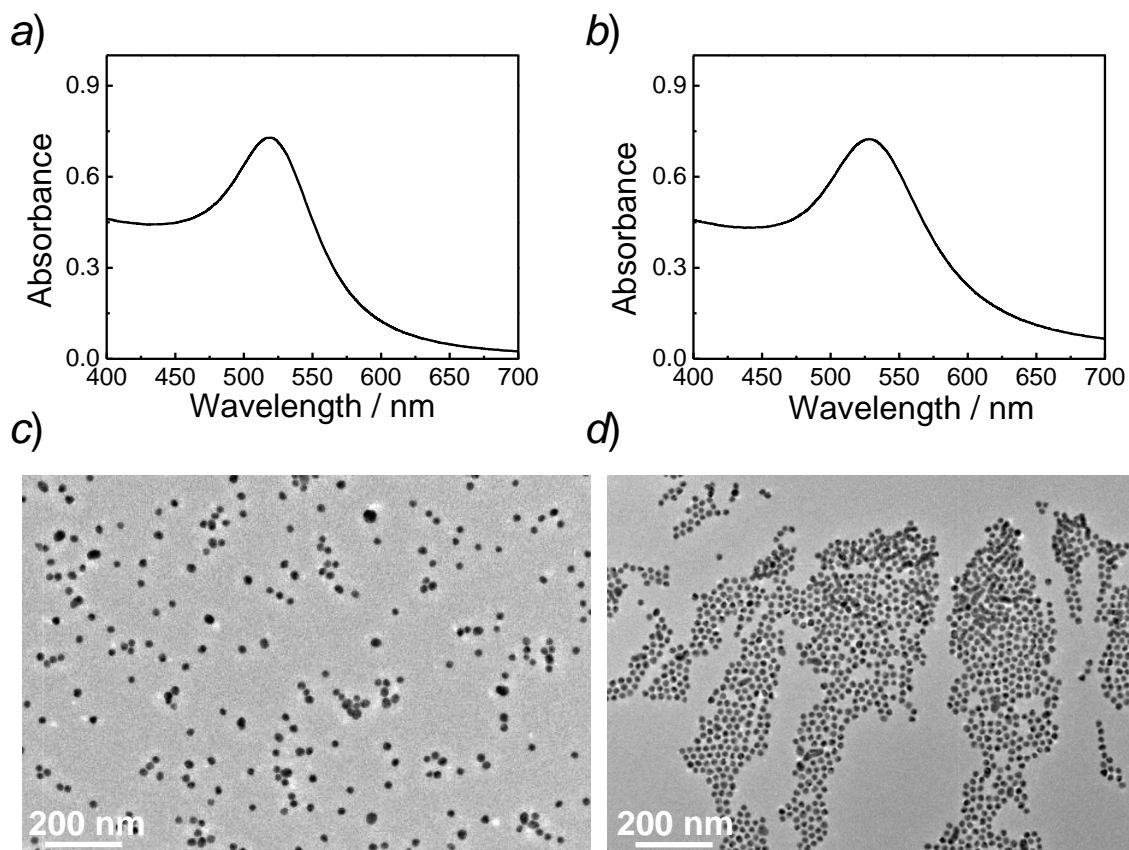


Figure S6. (a,b) UV/vis absorbance spectra and (c,d) TEM images of (a,c) aqueous dispersions of sodium citrate-stabilized AuNPs (~13 nm) and (b,d) hybrid **PV-2** amphiphiles in THF.

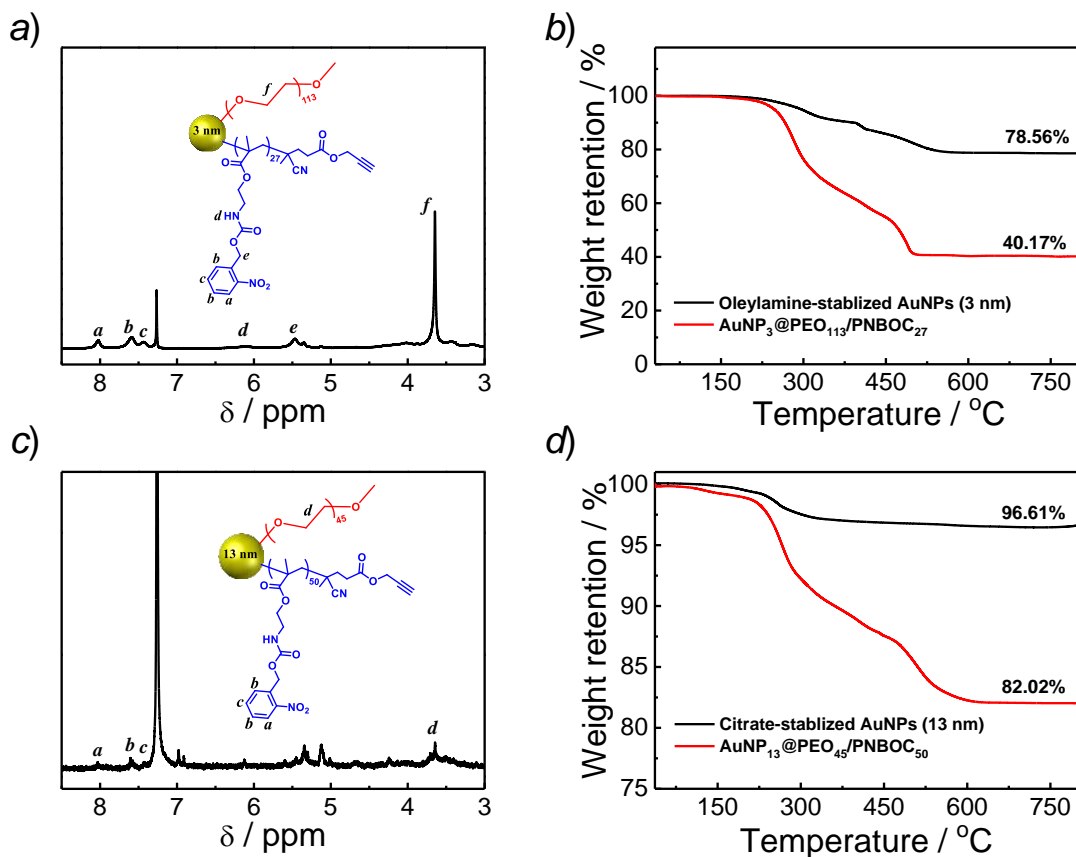


Figure S7. (a,c) ^1H NMR spectra recorded in CDCl_3 for hybrid (a) **PV-1** and (c) **PV-2** amphiphiles and (b,d) TGA profiles recorded for (b) OAm-stabilized AuNPs (~ 3 nm) and hybrid **PV-1** amphiphiles and (d) sodium citrate-stabilized AuNPs (~ 13 nm) and hybrid **PV-2** amphiphiles. TGA measurements were performed in air at a heating rate of 10 °C/min.

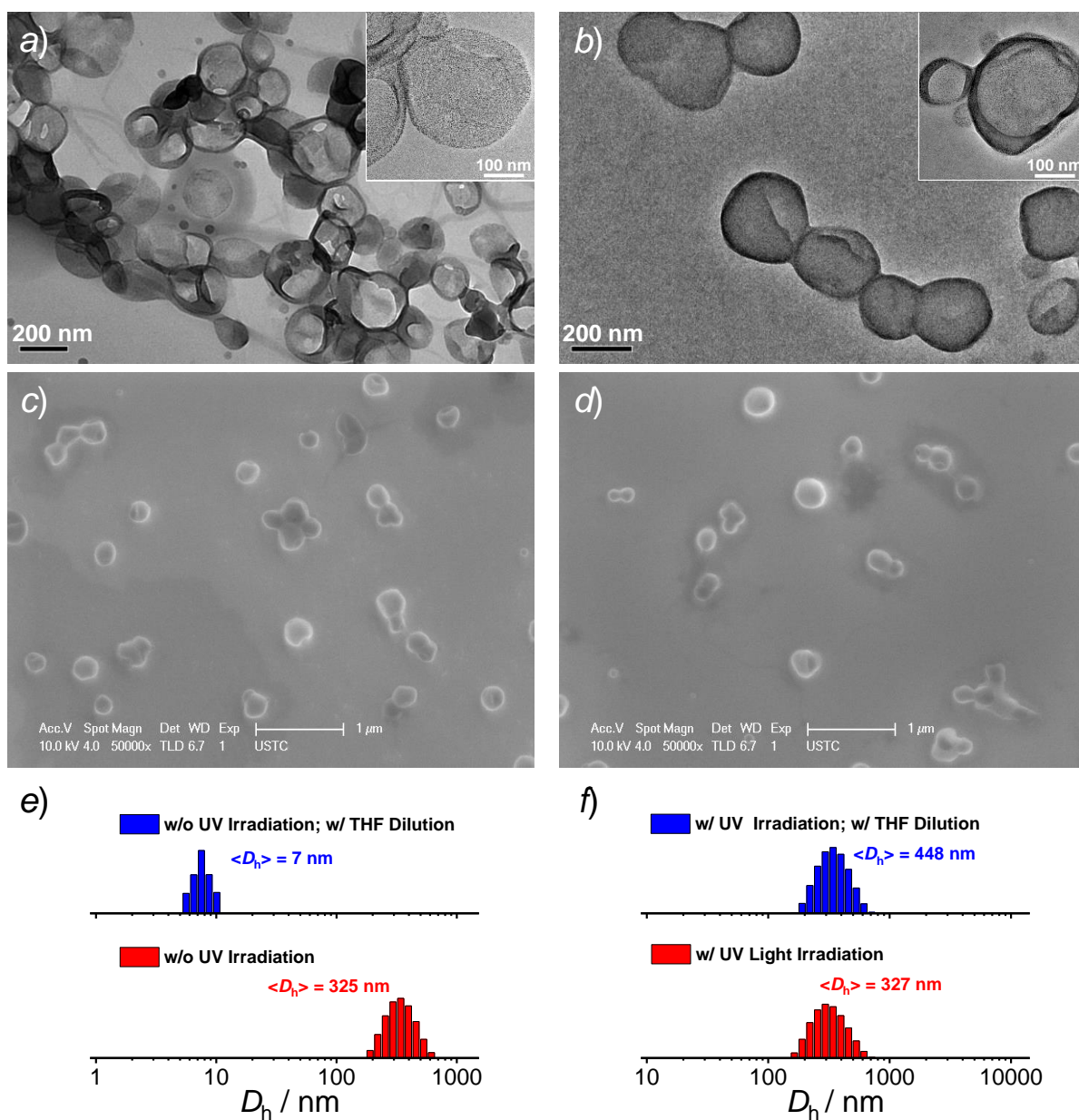


Figure S8. (a,b) TEM images and (c,d) SEM images recorded for **PV-1** vesicles (a,c) before and (b,d) after UV irradiation; the insets show enlarged hybrid vesicles. (e,f) Hydrodynamic distributions of (e) untreated and (f) UV-irradiated aqueous dispersions of **PV-1** vesicles before and after dilution with 9-fold THF. The vesicles were fabricated through a flash nanoprecipitation approach by quickly injecting THF dispersion of **PV-1** amphiphiles into deionized water in one shot.

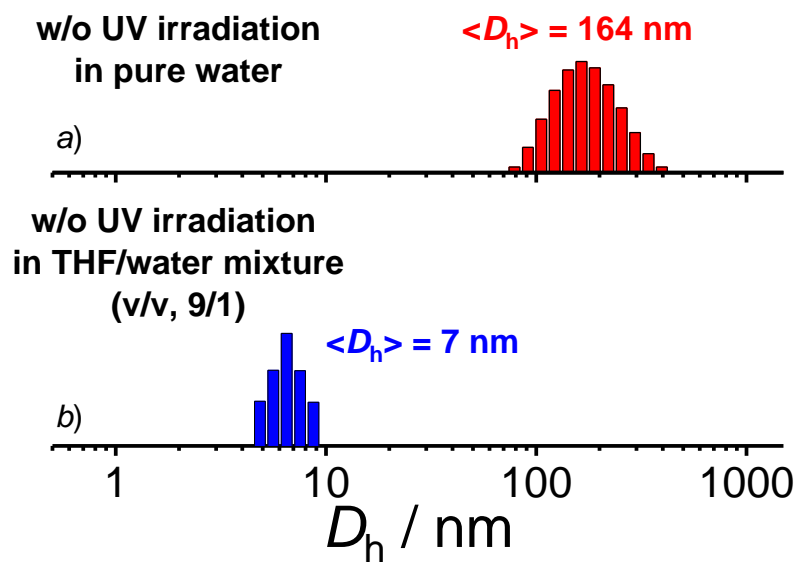


Figure S9. Hydrodynamic distributions, $f(D_h)$, of **PV-1** vesicles in (a) aqueous solution and (b) THF/water mixture (v/v, 9/1).

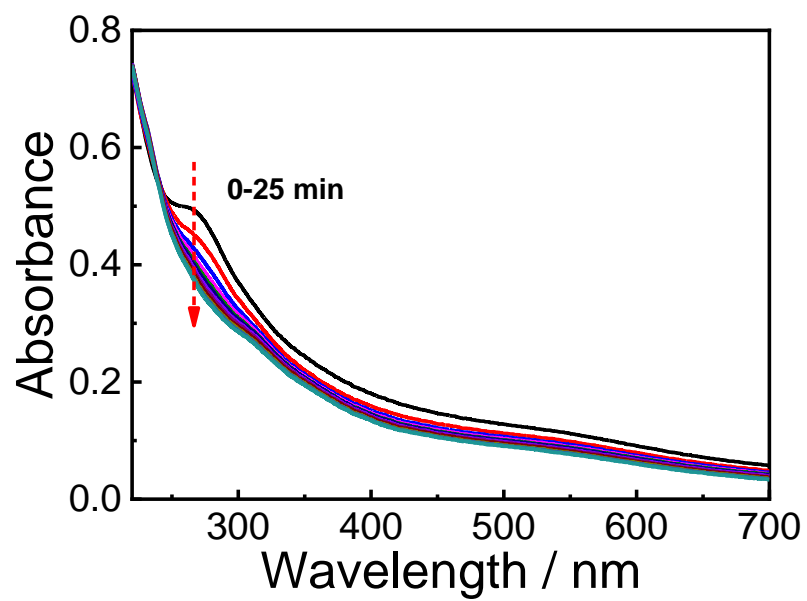


Figure S10. UV/vis absorbance spectra recorded for the aqueous dispersion of **PV-1** vesicles under 410 nm light irradiation ($\sim 1.0 \text{ W/cm}^2$).

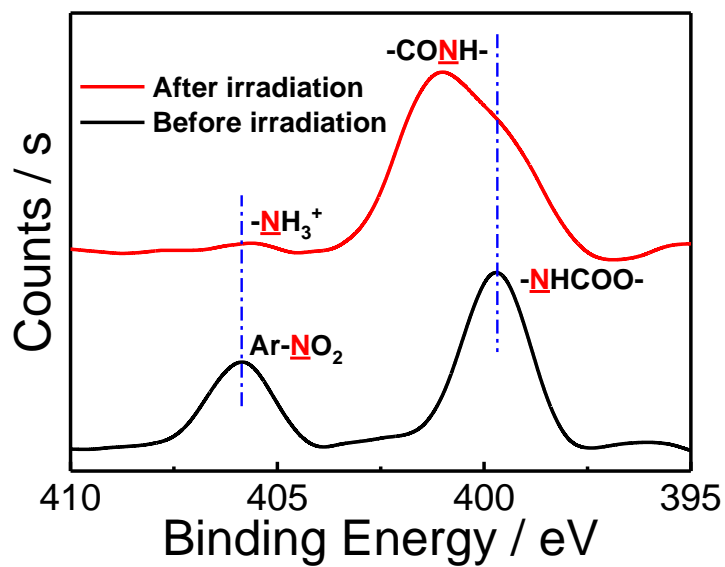


Figure S11. XPS N1s core-level spectra recorded for **PV-1** vesicles before and after UV (365 nm) irradiation (~ 1.0 mW/cm²). The samples were obtained via lyophilization of vesicle dispersions.

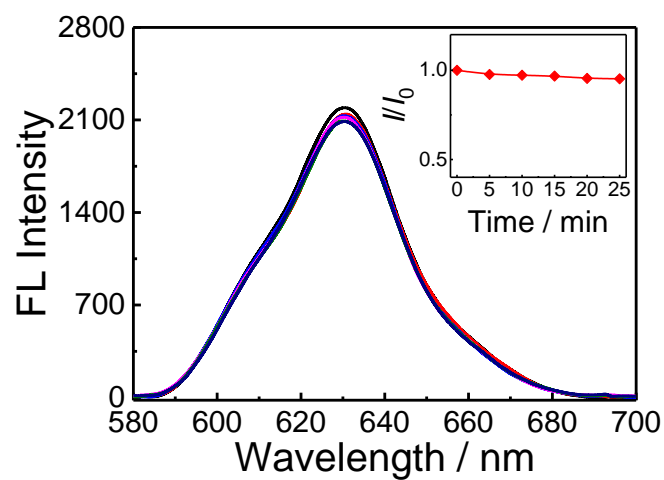


Figure S12. Fluorescence emission spectra ($\lambda_{\text{ex}} = 520 \text{ nm}$) of Nile red-loaded **PV-1** vesicles without light irradiation. The inset shows emission intensity changes at 630 nm.

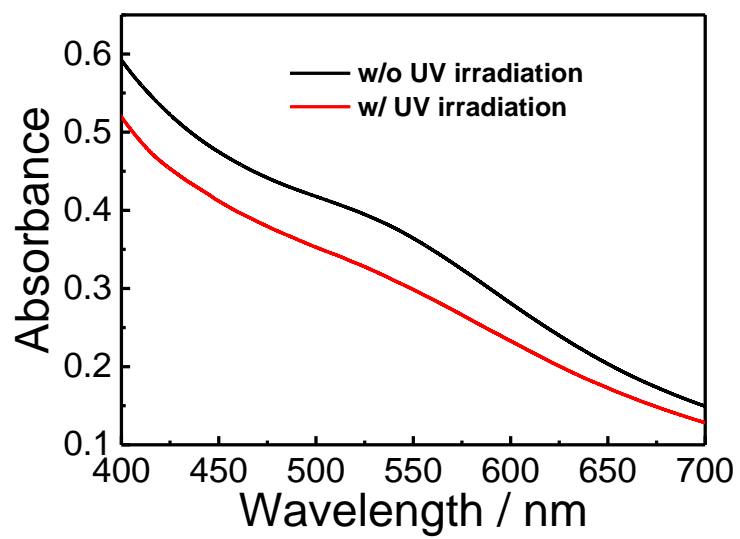


Figure S13. UV/vis spectra of untreated (black) and UV-irradiated (red) **PV-1** vesicles.

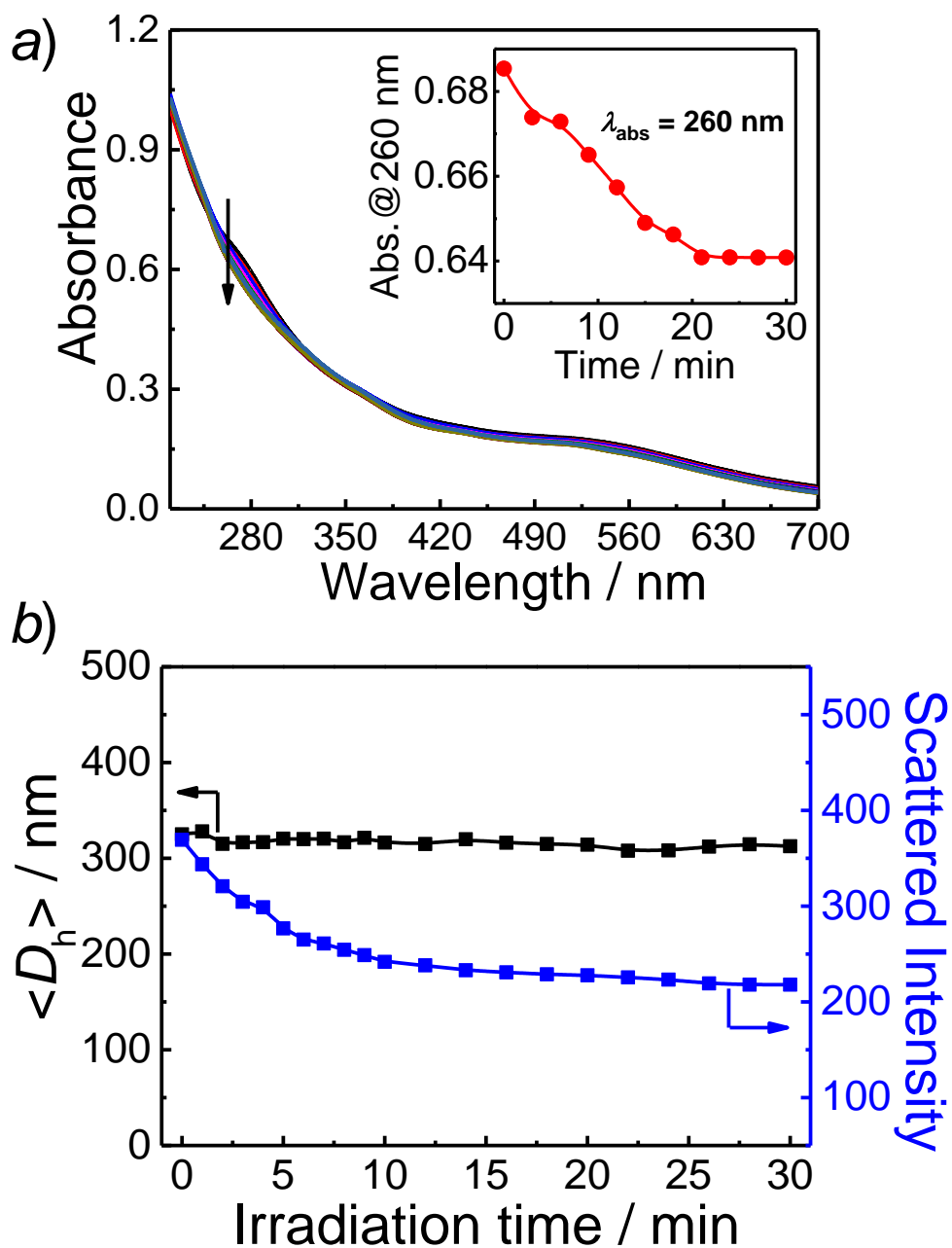


Figure S14. (a) Evolution of UV/vis absorbance spectra recorded for the aqueous dispersion of **PV-1** vesicles assembled from flash precipitation under UV (365 nm) irradiation. The inset shows absorbance changes at 260 nm under UV (365 nm) irradiation. (b) Evolution of intensity-average hydrodynamic diameters, $\langle D_h \rangle$, and scattered light intensities of **PV-1** vesicles under UV (365 nm) irradiation ($\sim 1.0 \text{ mW/cm}^2$).

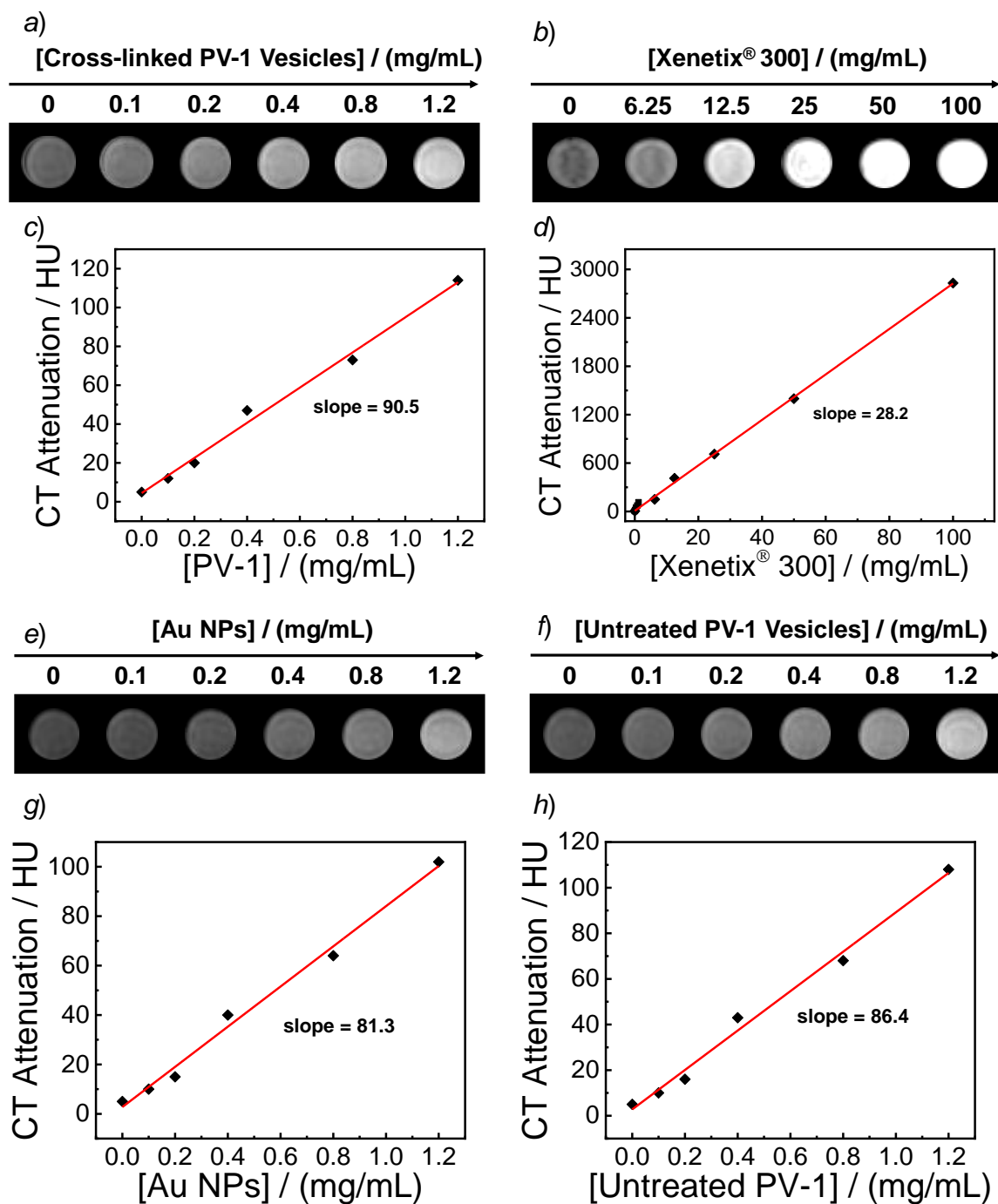


Figure S15. (a,b,e,f) CT phantom images and (c,d,g,h) HU measurements of the aqueous dispersions of (a,c) cross-linked **PV-1** vesicles, (b,d) commercially available Xenetix[®] 300, (e,g) CATB-stabilized Au NPs (~ 3 nm), and (f,h) untreated **PV-1** vesicles at varying concentrations.

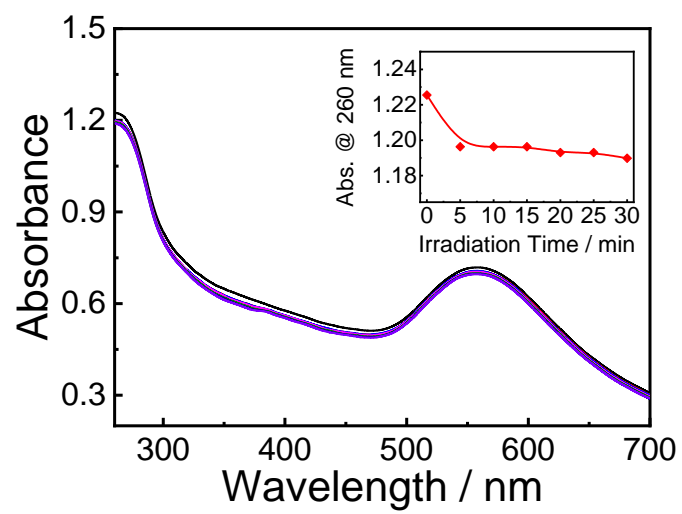


Figure S16. Evolution of UV/vis absorbance spectra recorded for the aqueous dispersion of **PV-2** vesicles under UV (365 nm) irradiation ($\sim 1.0 \text{ mW/cm}^2$). The inset shows corresponding absorbance changes at 260 nm.

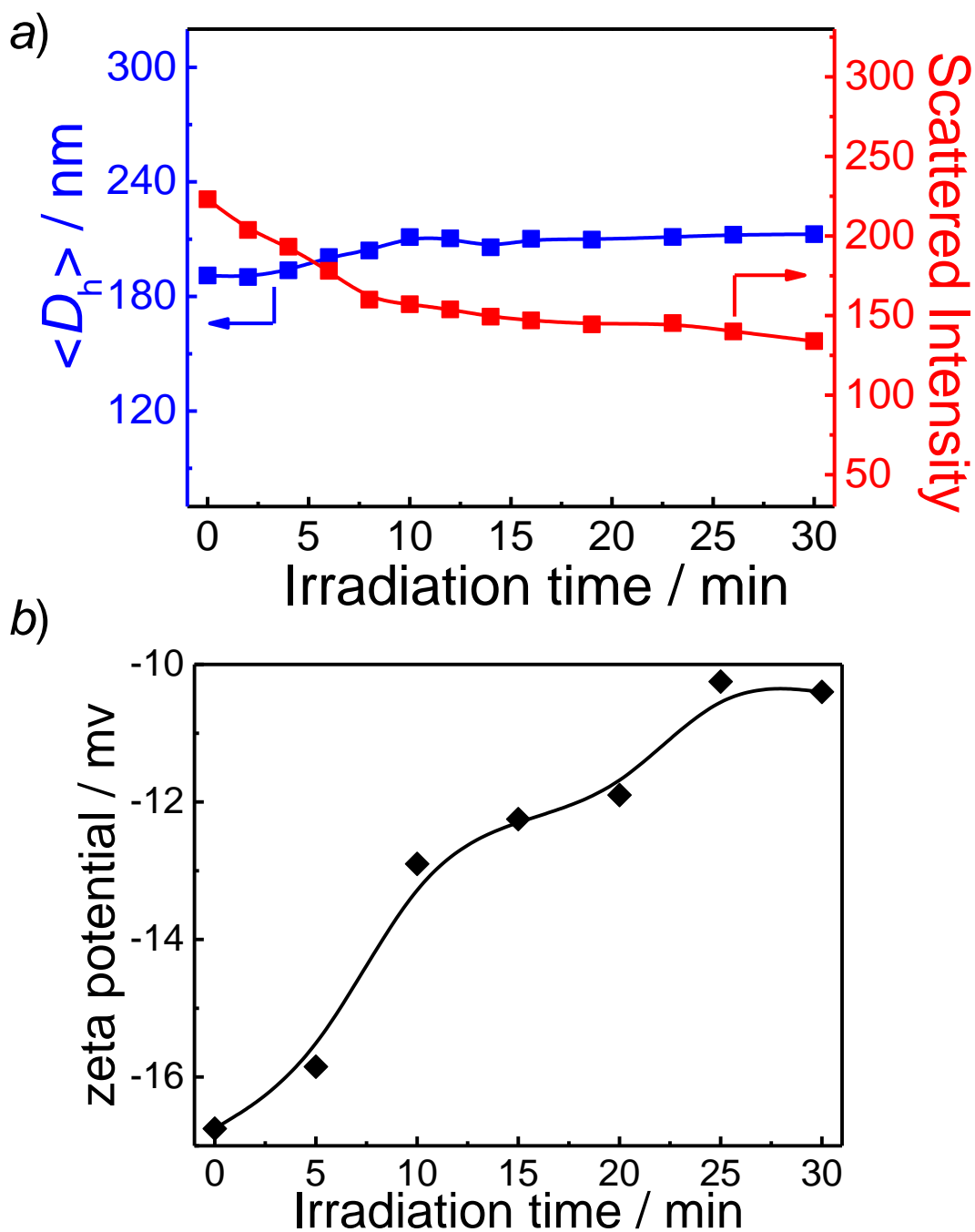


Figure S17. (a) Evolution of $\langle D_h \rangle$ and scattered light intensities of **PV-2** vesicles under UV (365 nm) light irradiation (~ 1.0 mW/cm²). (b) Irradiation duration-dependence of zeta potentials of **PV-2** vesicles under UV (365 nm) light irradiation (~ 1.0 mW/cm²).

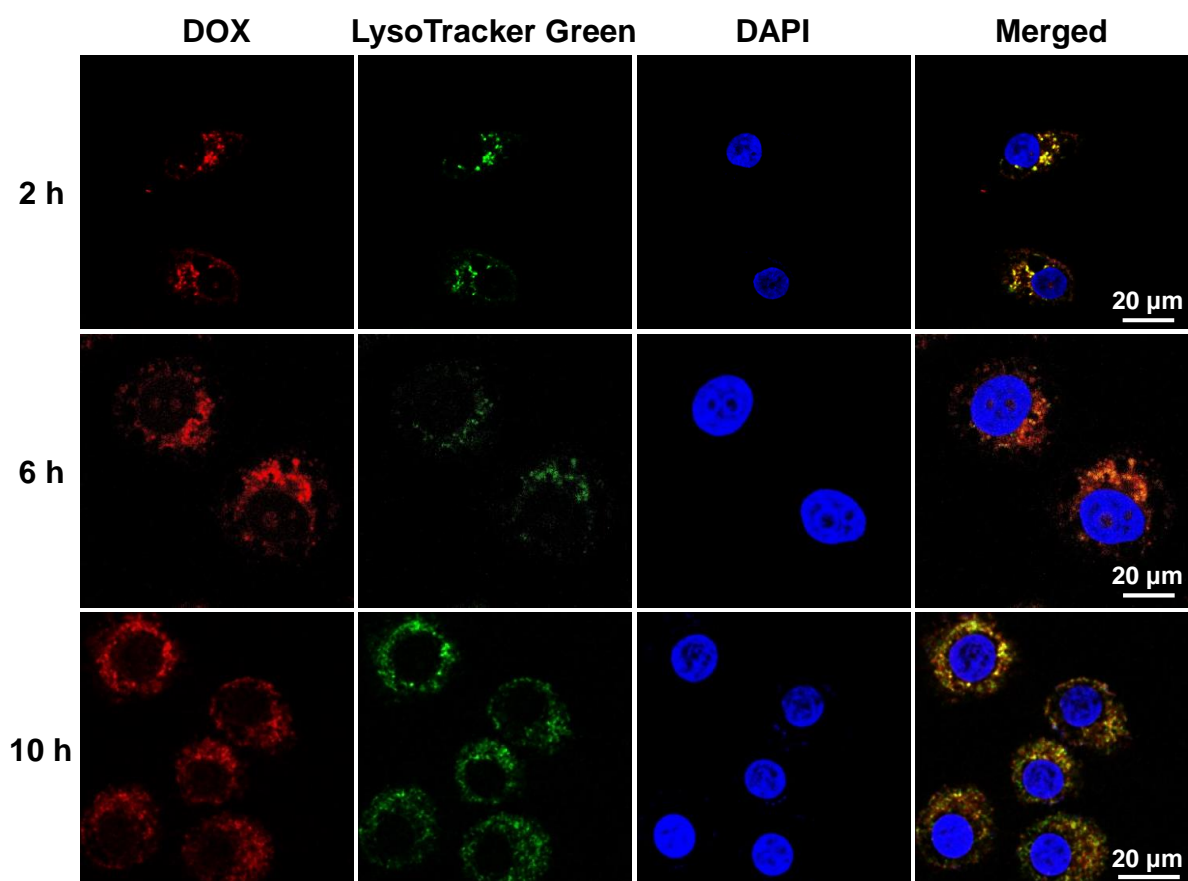


Figure S18. Representative CLSM images recorded for HepG2 cells after incubation with DOX and PTX co-loaded hybrid **PV-2** vesicles for 2 h, followed by rinsing with PBS buffer; the cells were further incubated for additional 2 h, 6 h, and 10 h, respectively. Late endosomes/lysosomes and cell nuclei were stained with LysoTracker green (green channel) and DAPI (blue channel), respectively.

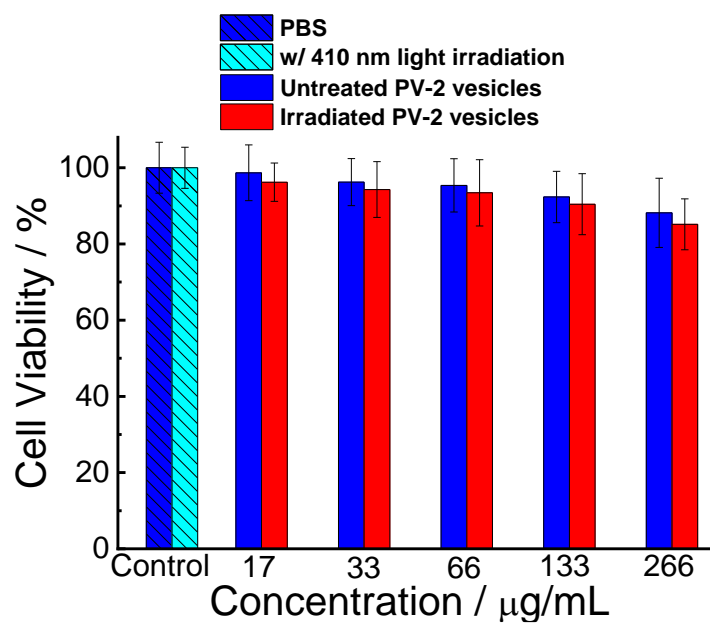


Figure S19. *In vitro* cytotoxicity of **PV-2** vesicles determined by MTT assay against HepG2 cells. The cells were incubated with **PV-2** vesicles without or with 410 nm light irradiation for 10 min at varying concentrations.

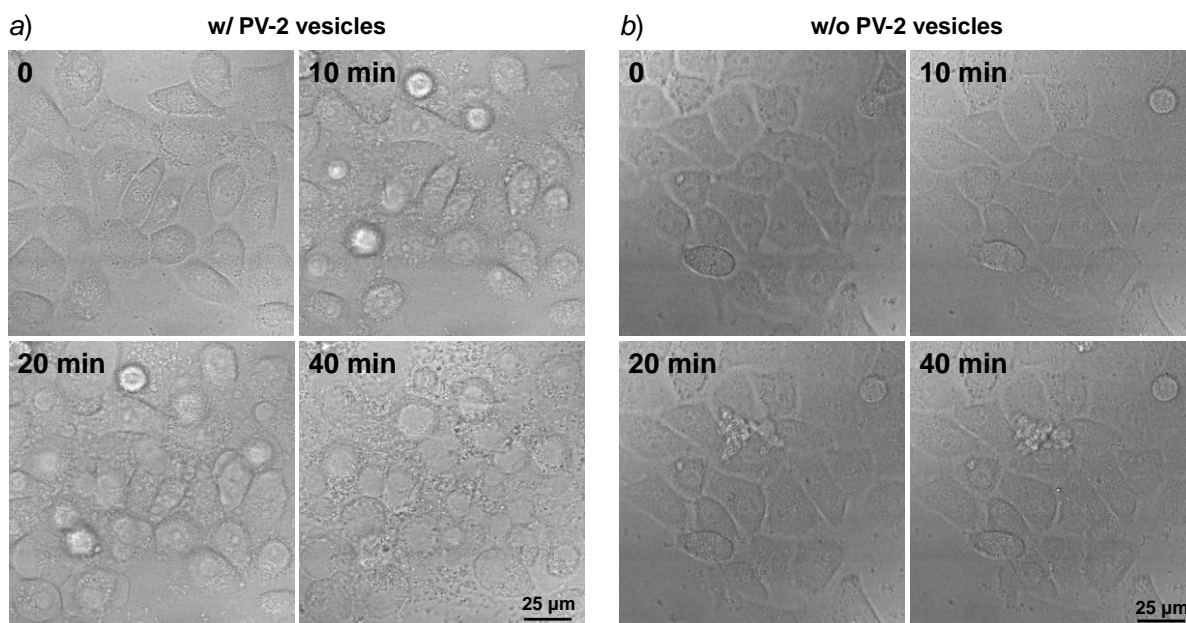


Figure S20. Changes in the morphologies of HepG2 cells incubated with (a) and (b) without pre-irradiated (365 nm; 10 min) **PV-2** vesicles for 2 h, followed by irradiation with a 543 nm laser ($\sim 0.8 \text{ W/cm}^2$) for varying durations (0-40 min).