Electronic Supplementary Material (ESI) for Materials Horizons.

Electronic Supplementary Information

Tumor-Adapting and Tumor-Remodeling AuNR@Dendrimer-Assemblies Nanohybrids Overcome Impermeable Multidrug-Resistant Cancer

Yachao Li, Xiao Zhang, Zhijun Zhang, Huayu Wu, Xianghui Xu, and Zhongwei Gu**

1. Experimental Section

1.1 Materials and Methods

Materials: Amino acids (Fmoc-Glu-OH, H-Glu-OtBu, Boc-Gly-OH, Boc-Pro-OH, Boc-Leu-OH NH_2-Gly-OMe Boc-Leu-OH, Boc-Ala-OH, Boc-Gly-OH) and condensation agents (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl), benzotriazole-1-yl-oxytripyrrolidino phosphonium hexafluorophosphate (PyBop) and 1-hydroxybenzotriazole hydrate (HOBT)) were purchased from GL Biochem. Ltd (Shanghai, China). Lipoic acid (LA), poly(ethylene glycol) methyl ether (mPEG), with the average molecular weight of 750, 3,3`-dithiodipropionic acid and DL-dithiothreitol (DTT) was purchased from Aladdin Reagents Company (Shanghai, China). N, N-diisopropylethylamine (DIPEA) and trifluoroacetic acid (TFA) were purchased from Asta Tech Pharmaceutical (Chengdu, China). Adriamycin (ADR) was purchased from Hisun Pharmaceutical (Zhejiang, China). Cell counting kit-8 (CCK-8), Hoechst 33342, 4',6-Diamidino-2-phenylindole and Apoptosis Detection Kit were purchased from Dojindo Laboratories (Japan). Cetyltrimethyl ammonium bromide (CTAB), silver nitrate, L-ascorbic acid, sodium borohydride, phenanthroline (MMPs inhibitor), buthionine sulfoximine (BSO) and glutathione monoester (GSH-OEt) were purchased from Sigma-Aldrich (USA). CD 31 and poly (ADP-ribose) polymerase (PARP) antibodys were purchased from Abcam (USA). Caspase-3 activity kit was purchased from Beyotime Biotechnology (China). CellEvent Caspase-3/7 red detection reagent was purchased from ThermoFisher scientific (C10423). 2`,7`-Dichlorofluorescein diacetate (DCF-DA), propidium iodide (PI) and fluorescein diacetate (FDA) were purchased from Sigma-Aldrich (USA). Apoptosis Detection Kits (Annexin V-PE / 7-AAD) was purchased from BD Biosciences (USA).

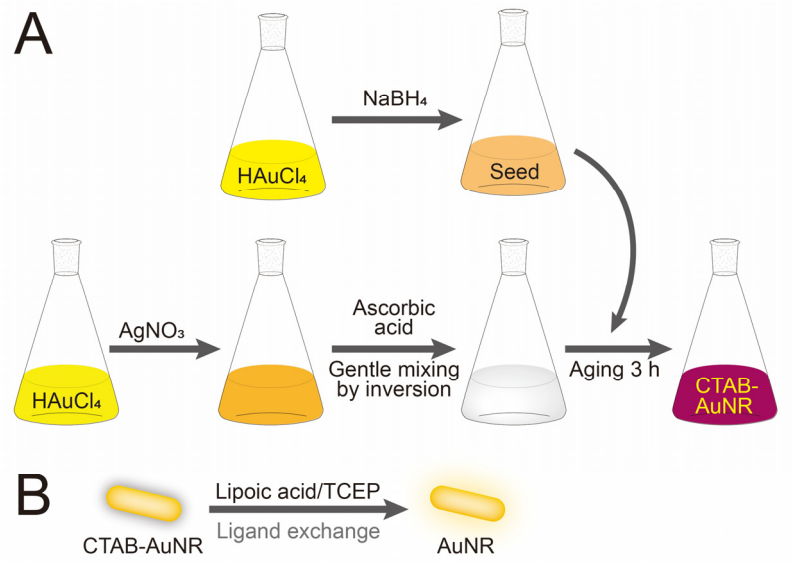
Chemoresistant human ovarian cancer cell lines (SKOV3/ADR cells) were obtained from West China School of Pharmacy Sichuan University (Chengdu, China). RPMI 1640 (modified) medium, penicillin, streptomycin, and fetal bovine serum (FBS) were purchased from Hyclone (USA). SKOV3/ADR cells were cultured in RPMI 1640 (modified) medium with 10% FBS and 1% penicillin/streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2.

Methods: ^1H NMR spectra were recorded on a Bruker Avance II NMR spectrometer at 400 MHz. The molecular weight of each compound was tested by matrix-assisted laser desorption ionization time of flight mass spectroscopy (MALDI-TOF MS, Bruker Autoflex III) or electrospray ionization time-of-flight mass spectrometry (ESI-TOF MS, Waters Q-TOF Premier, USA). The size and zeta potential were determined by a dynamic light scattering (DLS, Malvern NANO ZS, England). The nanostructures of nanoparticles were measured by transmission electron microscope (TEM, FEI Tecnai GF20S-TWIN, USA) and atomic force

microscopy (AFM, MFP-3D-BIO, USA). The cell viability, hexokinase activity and ATP level were measured using a Thermo Varioskan Flash microplate reader (USA). CRI (CRi Maestro EX, USA) *in vivo* imaging system (IVIS) was used for *in vivo* fluorescence imaging. MSOT imaging system (MSOT in Vision 128, iTheramediacal, Germany) was used for *in vivo* photoacoustic (PA) imaging. The cryosections of MCF-7R tumors and cells were imaged using two-photon laser scanning microscopy (2PLSM, Nikon A1R MP+, Japan) or confocal laser scanning microscopy (CLSM, Leica SP5, Germany). The treated tissue slices were imaged using inverted optical/fluorescence microscopy (Leica DMI4000B, Germany). The amount of Au was measured by atomic absorption spectrophotometry (AAS, VARIAN, SpectrAA 220Z, USA)

1.2 Synthesis of AuNR

Au nanorods were synthesized by seed-mediated approach.



Scheme S1. (A) Scheme of the seed-mediated growth method to synthesize CTAB-AuNR. (B) AuNR surface modification via ligand exchange reaction.

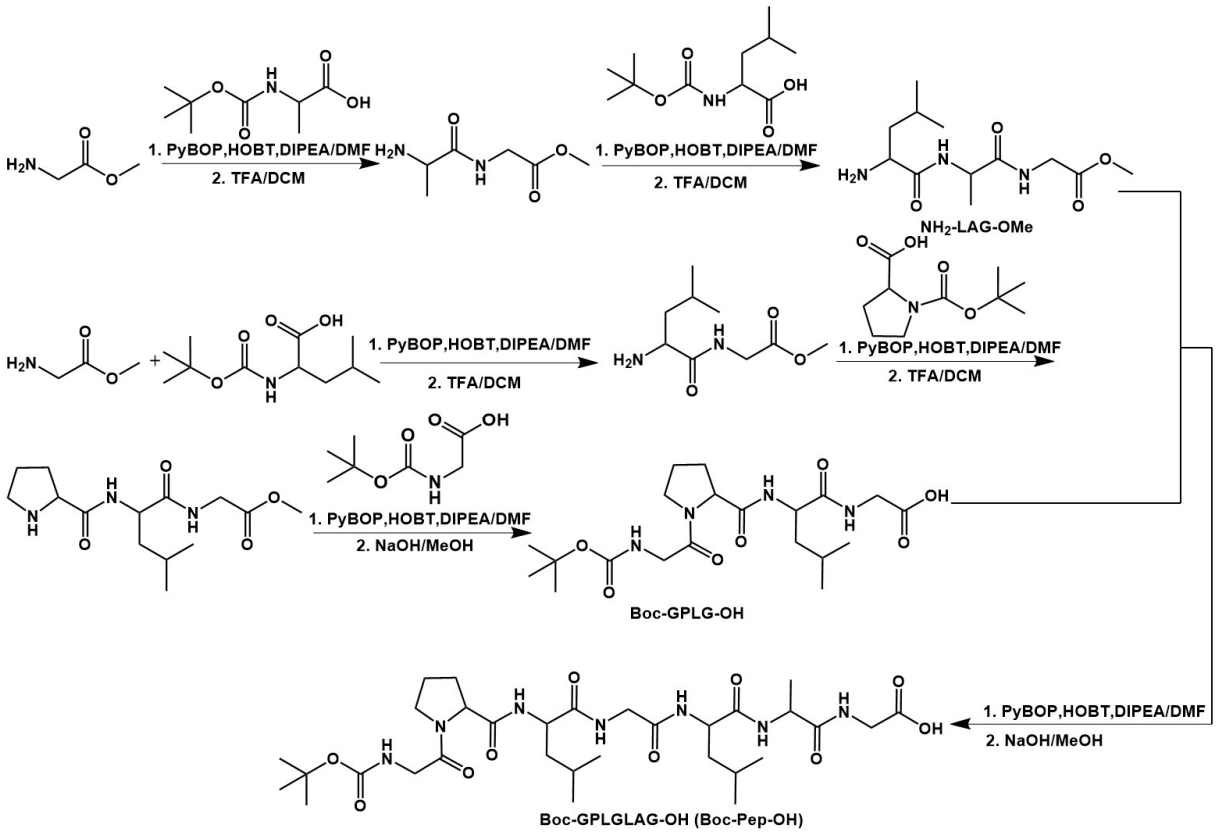
Preparation of Au seed. $HAuCl_4$ solution (0.01M, 0.25 mL) added to CTAB solution (0.10 M, 7.5 mL) and mixed gently. Then $NaBH_4$ solution (0.01 M, 0.60 mL) was added to the mixture and stored at 25 ℃ for 2 h.

Preparation of Au nanorods. Cetyltrimethylammonium bromide (CTAB, 0.1 M), $HAuCl_4$ (1.0×10^{-4} M) and $AgNO_3$ (6.0×10^{-5} M) were added orderly with a volume of 500 mL and gently mixed. Then L-ascorbic acid (1.6×10^{-5} M) was added and mixed. Finally, Au seed (5.0×10^{-7} M) was added to the mixture and mixed for 10 s. The solution was left undisturbed overnight. The solution was centrifuged for 30 min at a speed of 12 000 rpm to precipitate the CTAB stabilized Au nanorods (CTAB-AuNR). Then lipoic acid and TCEP were dissolved in

water at pH 8 at a lipoic acid: TCEP molar ratio of 1: 1. Then the mixture was stirred for 1 h with UV irradiation. Au nanorods were added to the solution at a lipoic acid: Au molar ratio of 1: 10 and stirred for 24 h. The solution centrifuged for 30 min at a speed of 12 000 rpm. The precipitate was reacted with fresh lipoic acid ligand as above for twice. The excessive lipoic acid was removed by centrifugation. Then the precipitate was lyophilized to obtain AuNR.

1.3 Synthesis of Dendrimers

1.3.1 Synthesis of MMP-2 sensitive peptide (H-GPLGLAG-OH)



Scheme S2 Synthetic route of Boc-GPLGLAG-OH

Synthesis of Boc-Ala-Gly-OMe

H-Gly-OMe (G, 5g), Boc-Ala-OH (A, 1 equiv with respect to H-Gly-OMe), PyBOP (1.5 equiv with respect to carboxyl group), HOBT (1 equiv with respect to carboxyl group) were dissolved in 30 mL anhydrous N, N-dimethylformamide (DMF) in N_2 atmosphere. N, N-diisopropylethylamine (DIPEA) was then added in ice-bath. And the reaction mixture stirred for 24 h at room temperature. After DMF removed, chloroform was added and the mixture was washed with saturated NaCl, $NaHCO_3$ and 1 M HCl solution time and again. The solution was dried with $MgSO_4$ overnight. After chloroform removed, the residue was purified by a silica column using 100% DCM as an eluent and the product was obtained as a white solid in 89%

yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42 (s, 1H), 5.67 (s, 1H), 4.31 (m, 1H), 4.16 (s, 2H) 3.74 (s, 3H), 1.44 (s, 9H), 1.47 (d, 3H). MS (ESI-TOF) (m/z) calcd. $[M+H]^+$ calcd for $C_{11}H_{21}N_2O_5$, 207.14, found 207.06.

Synthesis of Boc-Leu-Ala-Gly-OMe

Boc-Ala-Gly-OMe was dissolved in anhydrous DCM and treated with trifluoroacetic acid (TFA, 10 equiv with respect to tert-butoxycarbonyl groups) in ice-bath. The mixture was stirred for 12 h. The solution was concentrated and then precipitated in excess ethyl ether to obtain H-Ala-Gly-OMe. $[M+Na]^+$ calcd for $C_6H_{12}N_2O_3Na$, 182.07, found 182.34. H-Ala-Gly-OMe, Boc-Leu-OH (L, 1 equiv with respect to H-Ala-Gly-OMe), PyBOP (1.5 equiv with respect to carboxyl group) and HOBT (1 equiv with respect to carboxyl group) were dissolved in 30 mL anhydrous DMF under N_2 atmosphere. DIPEA was then added. And the reaction mixture was stirred for 24 h at room temperature. The product was purified by washing and silica column to get a white solid in 83% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.9 (s, 1H), 6.69 (s, 1H), 4.96-4.85 (m, 2H), 4.53 (t, 1H), 4.13-3.93 (m, 3H), 3.74 (s, 3H), 1.75 (t, 2H), 1.52-1.48 (m, 1H), 1.44 (s, 9H), 0.95 (t, 6H). MS (ESI-TOF) (m/z) calcd. $[M+Na]^+$ calcd for $C_{17}H_{31}N_3O_6Na$, 396.21, found 396.04.

Synthesis of Boc-Leu-Gly-OMe

H-Gly-OMe (5 g), Boc-Leu-OH (1 equiv with respect to H-Gly-OMe), PyBOP (1.5 equiv with respect to carboxyl group), HOBT (1 equiv with respect to carboxyl group) were dissolved in 30 mL anhydrous N, N-dimethylformamide (DMF). After the reaction mixture was stirred at 0 ℃ for 30 min in N_2 atmosphere, N, N-diisopropylethylamine (DIPEA) was then added. And the reaction mixture was stirred for 24 h at room temperature. The product was purified by washing and silica column to get a white solid in 88% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.61 (s, 1H), 4.94-4.73 (m, 1H), 4.16 (s, 1H), 4.06 (s, 2H) 3.76 (s, 3H), 1.75-1.65 (m, 2H), 1.54-1.48 (m, 1H), 1.45 (s, 9H), 0.95 (t, 6H). MS (ESI-TOF) (m/z) calcd. $[M+H]^+$ calcd for $C_{14}H_{27}N_2O_5$, 303.37, found 303.03.

Synthesis of Boc-Pro-Leu-Gly-OMe

Boc-Leu-Gly-OMe was dissolved in anhydrous DCM and treated with trifluoroacetic acid (TFA, 10 equiv with respect to tert-butoxycarbonyl groups) in ice-bath. The mixture was stirred for 12 h. The solution was concentrated and then precipitated in excess ethyl ether to obtain H-Leu-Gly-OMe. $[M+H]^+$ calcd for $C_9H_{19}N_2O_3$, 203.14, found 203.03. H-Leu-Gly-OMe, Boc-Pro-OH (P, 1 equiv with respect to H-Ala-Gly-OMe), PyBOP (1.5 equiv with respect to carboxyl group) and HOBT (1 equiv with respect to carboxyl group) were dissolved in 30 mL anhydrous N, N-dimethylformamide (DMF). After the reaction mixture was stirred at 0 ℃ for

30 min in N_2 atmosphere, N, N-diisopropylethylamine (DIPEA) was then added. And the reaction mixture was stirred for 24 h at room temperature. The product was purified by washing and silica column to get a white solid in 78% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.09 (s, 1H), 7.96 (s, 1H), 4.54-4.43 (m, 1H), 4.29 (s, 1H), 4.16-3.83 (m, 2H), 3.73 (s, 3H), 3.53-3.30 (m, 2H), 2.34-1.75 (m, 6H), 1.46 (s, 10H), 0.93 (d, 6H). MS (ESI-TOF) (m/z) calcd. $[M+H]^+$ calcd for C19H34N3O6, 400.24, found 400.04.

Synthesis of Boc-Gly-Pro-Leu-Gly-OMe

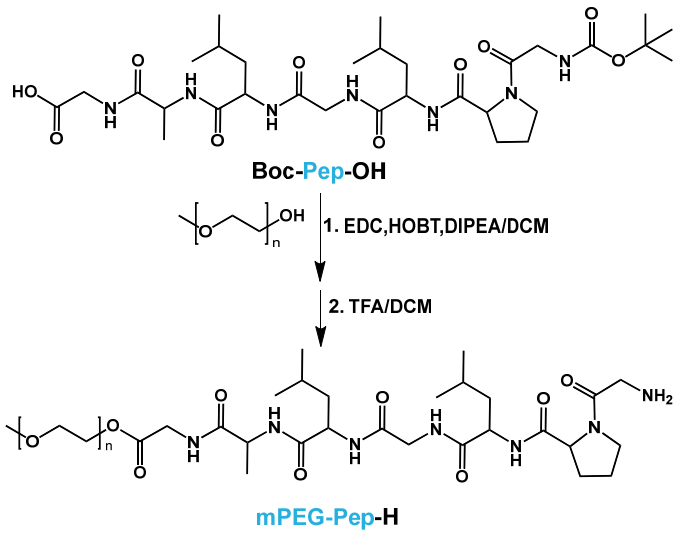
Boc-Pro-Leu-Gly-OMe was dissolved in anhydrous DCM and treated with trifluoroacetic acid (TFA, 10 equiv with respect to tert-butoxycarbonyl groups) in ice-bath. The mixture was stirred for 12 h. The solution was concentrated and then precipitated in excess ethyl ether to obtain H-Pro-Leu-Gly-OMe. $[M+H]^+$ calcd for C14H26N3O4, 300.18, found 300.03. H-Pro-Leu-Gly-OMe, Boc-Gly-OH (1 equiv with respect to H-Pro-Leu-Gly-OMe), PyBOP (1.5 equiv with respect to carboxyl group) and HOBT (1 equiv with respect to carboxyl group) were dissolved in 30 mL anhydrous N, N-dimethylformamide (DMF). After the reaction mixture was stirred at 0 ℃ for 30min in N_2 atmosphere, N, N-diisopropylethylamine (DIPEA) was then added. And the reaction mixture was stirred for 24 h at room temperature. The product was purified by washing and silica column to get a white solid in 73% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.01 (s, 1H), 6.92 (d, 1H), 5.43 (s, 1H), 4.58-4.44 (m, 2H),4.14 (d, 1H), 4.09 (d, 1H), 3.96 (d, 2H), 3.75 (s, 3H), 3.66-3.59 (m, 2H), 2.14-1.99 (m, 4H), 1.63-1.52 (m, 2H), 1.47-1.41 (m, 10H), 0.92 (d, 6H). MS (ESI-TOF) (m/z) calcd. $[M+H]^+$ calcd for C21H37N4O7, 457.26, found 457.07.

Synthesis of Boc-Gly-Pro-Leu-Gly-Leu-Ala-Gly-OH (Boc-GPLGLAG-OH / Boc-Pep-OH)

Prepare sodium hydroxide in methanol (NaOH–MeOH) solution (1M). Boc-Gly-Pro-Leu-Gly-OMe was treated with NaOH–MeOH solution (1M) and stirred for 12 h. After removal of MeOH, ethyl acetate was added. The mixture was then treated with 1M HCl solution until pH 7. And the organic phase dried in anhydrous Na_2SO_4 overnight. After ethyl acetate removed, yellow oily liquid Boc-Gly-Pro-Leu-Gly-OH was obtained in 67% yield. MS (ESI-TOF) (m/z) calcd. $[M+H]^+$ calcd for C20H35N4O7, 443.24, found 443.15. Boc-Leu-Ala-Gly-OMe was dissolved in anhydrous DCM and treated with trifluoroacetic acid (TFA, 10 equiv with respect to tert-butoxycarbonyl groups) in ice-bath. The mixture was stirred for 12 h. The solution was concentrated and then precipitated in excess ethyl ether to obtain H-Leu-Ala-Gly-OMe. MS (ESI-TOF) (m/z) calcd. $[M+H]^+$ calcd for C12H24N3O4, 274.17, found 274.04. H-Leu-Ala-Gly-OMe, Boc-Gly-Pro-Leu-Gly-OH (1 equiv with respect to H-Leu-Ala-Gly-OMe), PyBOP (1.5 equiv with respect to carboxyl group), HOBT (1 equiv with respect to carboxyl group) were dissolved in 30 mL anhydrous N, N-dimethylformamide (DMF). After the reaction

mixture was stirred at 0 ℃ for 30 min in N_2 atmosphere, N, N-diisopropylethylamine (DIPEA) was then added. And the reaction mixture stirred for 24 h at room temperature. The product was purified by washing and silica column to get a white solid in 68% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (s, 1H), 7.20-7.06 (m, 4H), 5.42 (s, 1H), 4.51 (t, 1H), 4.31 (t, 1H), 4.15-3.74 (m, 6H), 3.72 (s, 3H), 3.61-3.54 (m, 2H), 2.13-2.02 (m, 4H), 1.87-1.80 (m, 4H), 1.78-1.69 (m, 4H), 1.46 (d, 3H), 1.43 (s, 9H), 1.00-0.86 (m, 12H). MS (ESI-TOF) (m/z) calcd. $[M+H]^+$ calcd for $C_{32}H_{56}N_7O_{10}$, 698.40, found 698.04. Boc-Pep-OMe was dissolved in anhydrous DCM and treated with trifluoroacetic acid (TFA, 10 equiv with respect to tert-butoxycarbonyl groups) in ice-bath. The mixture was stirred for 12 h. The solution was concentrated and then precipitated in excess ethyl ether to obtain MS (ESI-TOF) (m/z) calcd. $[M-H]^-$ calcd for $C_{31}H_{52}N_7O_{10}$, 682.39, found 681.98.

1.3.2 Synthesis of mPEG-Pep-H

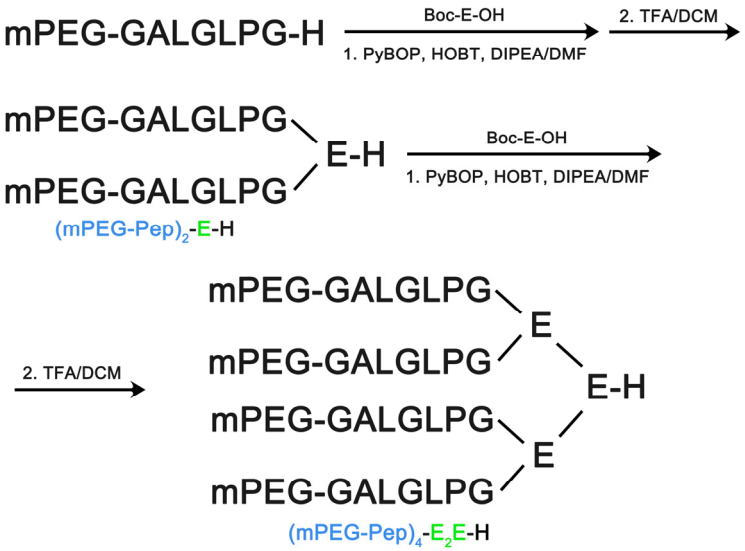


Scheme S3. Synthetic route of mPEG-Pep-H.

OH-Pep-Boc (1.00 g, 1.57 mmol), mPEG (1.76 g), HOBT (0.32 g, 2.35 mmol) and EDC.HCl (0.45 g, 2.35 mmol) were dissolved in anhydrous dichloromethane (DCM) with nitrogen protection, and DIPEA (1.05 mL, 6.25 mmol) was added, subsequently. The reaction was stirred for 24 h at room temperature. DCM was removed by vacuum evaporation and the mixture was dissolved in chloroform. Then the mixture was washed with saturated NaCl, saturated $NaHCO_3$ and HCl (1 M). The solution was dried with anhydrous $MgSO_4$ for 12 h, and then purified by column chromatography to obtain mPEG-GALGLPG-Boc. mPEG-GALGLPG-Boc was treated with TFA and then precipitated in excess ice anhydrous ethyl ether to obtain mPEG-GALGLPG-H. (yield: 69%) ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.65 (m, br, CH_2CH_2O), 3.83 (s, OCH_3), 2.08 (m, CH_2), 1.83 (m, CH_3). MALDI-TOF-MS analysis of mPEG-GALGLPG-Boc showed a broad peak with a center of mass around 1312.7, corresponding to theoretical mass. And the distance between neighboring peaks was

corresponding to the mass of a PEG repeat unit with 44 Da.

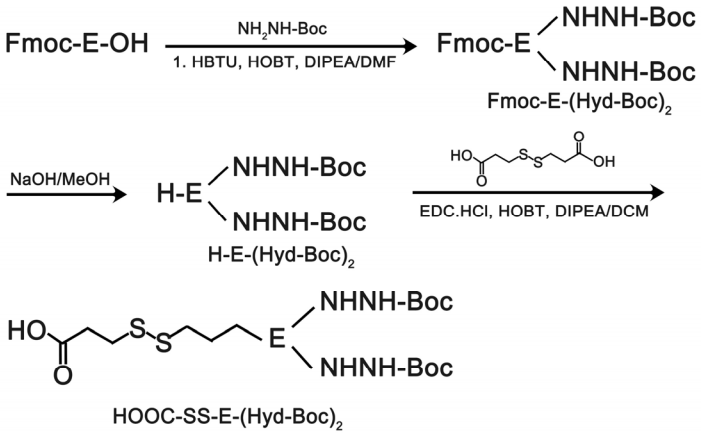
1.3.3 Synthesis of (mPEG-Pep)$_4$-E$_2$E-H dendrimers



Scheme S4. Synthetic route of (mPEG-Pep)$_4$-E$_2$E-H dendrimers.

Boc-Glu-OH (E, 100.00 mg, 0.40 mmol), PyBOP (624.48 mg, 1.20 mmol), HOBT (118.91 mg, 0.88 mmol) and H-Pep-mPEG (1.38 g) were dissolved in 35 mL anhydrous DMF with nitrogen protection. DIPEA (0.79 mL, 4.80 mmol) was then added in ice bath. And the reaction mixture stirred for 36 h at room temperature. The product was purified by washing and silica column (SiO_2) with DCM/MeOH = 20: 1 as described above to get a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.15 (s, CH_2), 3.58 (m, br, CH_2CH_2O), 3.75 (s, OCH_3), 2.01 (m, CH_2), 1.83 (m, CH_3). MALDI-TOF-MS analysis of Boc-E-(Pep-mPEG)$_2$ showed a broad peak with a center of mass around 2147.3, corresponding to theoretical mass. And the distance between neighboring peaks was corresponding to the mass of a PEG repeat unit with 44 Da. Then Boc-E-(Pep-mPEG)$_2$ (1.50 g) was dissolved in anhydrous DCM and treated with TFA (0.6 mL, 3.76 mmol) in ice-bath. The mixture was stirred for 12 h. The solution was concentrated and then precipitated in excess ice anhydrous ethyl ether to obtain H-E-(Pep-mPEG)$_2$ dendrimers. (yield: 69%) The synthesis step of H-E-(Pep-mPEG)$_2$ repeated to obtain (mPEG-Pep)$_4$-E$_2$E-H. (yield: 63%) ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.31-4.20 (m, CH), 3.82 (m, CH_2), 3.64 (m, CH_2CH_2O), 3.38 (s, OCH_3), 1.43 (d, CH_3), 0.89 (d, CH_3). MALDI-TOF-MS analysis of Boc-E$_2$E-(Pep-mPEG)$_4$ showed a broad peak with a center of mass around 4298.9, corresponding to theoretical mass. And the distance between neighboring peaks was corresponding to the mass of a PEG repeat unit with 44 Da.

1.3.4 Synthesis of HOOC-SS-E-(Hyd-Boc)$_2$



Scheme S5. Synthetic route of HOOC-SS-E-(Hyd-Boc)$_2$.

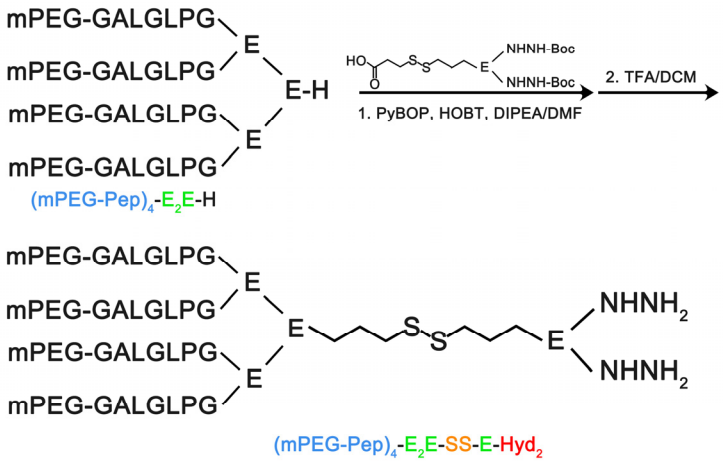
Synthesis of NH$_2$-E-(Hyd-Boc)$_2$

Fmoc-E-OH (2.00 g, 4.41 mmol), tert-Butyl carbazate (1.75 g, 13.23 mmol), HBTU (5.02 g, 13.23 mmol) and HOBT (1.79 g, 13.23 mmol) were dissolved in anhydrous DMF and stirred at 0 ℃ for 30 min with nitrogen protection. Then DIPEA (8.74 mL, 52.92 mmol) was added and remove the ice bath. Then the reaction was stirred for 48 h at room temperature. The product was purified as above to get Fmoc-E-(Hyd-Boc)$_2$ in 78% yield. ^{1}H NMR (400 MHz, DMSO-*d6*): δ 9.70 (s, 1H), 9.51 (s, 1H), 8.80 (s, 1H), 8.69 (s, 1H), 7.95 (s, 1H), 7.92-7.27 (m, 8H), 4.23 (d, 2H), 4.05-3.97 (m, 2H), 2.89 (t, 2H), 2.73 (t, 2H), 1.40 (s, 18H). MS (ESI-TOF) (m/z) calcd. [M+Na]$^+$ calcd for C30H39N5O8Na, 620.27, found 620.07. Fmoc-E-hyd-Boc$_2$ was treated with NaOH–MeOH solution (1M, 5 equiv of Fmoc groups) and stirred for 4 h. After removal of MeOH, deionized water was added. The product was extracted by ethyl acetate several times. Then the organic phase dried by anhydrous Na_2SO_4 overnight. Removal of ethyl acetate to afford a white solid H-E-hyd-Boc$_2$. (62% yield) [M+H]$^+$ calcd for C15H30N5O6, 376.21, found 376.13.

Synthesis of HOOC-SS-E-(Hyd-Boc)$_2$

H-E-(Hyd-Boc)$_2$ (1.00 g, 2.67 mmol), 3,3`-dithiodipropionic acid (0.56 g, 2.67 mmol), EDC.HCl (0.51 g, 2.67 mmol) and HOBT (0.36 g, 2.67 mmol) were dissolved in anhydrous DMF and stirred at 0 ºC for 30 min with nitrogen protection. Then DIPEA (2.6 mL, 16.02 mmol) was added and remove the ice bath. The reaction was stirred for 24 h at room temperature. After removal of DCM, the product was purified by washing and silica column (SiO_2) to get a white solid. (yield: 71%) ^{1}H NMR (400 MHz, CDCl3): δ 9.84 (s, 1H), 9.66 (s, 1H), 7.07 (s, 1H), 6.87 (s, 1H), 6.47 (s, 1H), 4.54 (t, 1H), 2.95 (m, 4H), 2.71 (m, 4H), 2.12 (m, 4H), 1.48 (s, H). MS (ESI-TOF) (m/z) calcd. [M-H]$^-$ calcd for C21H36N5O9S2, 566.20, found 566.04.

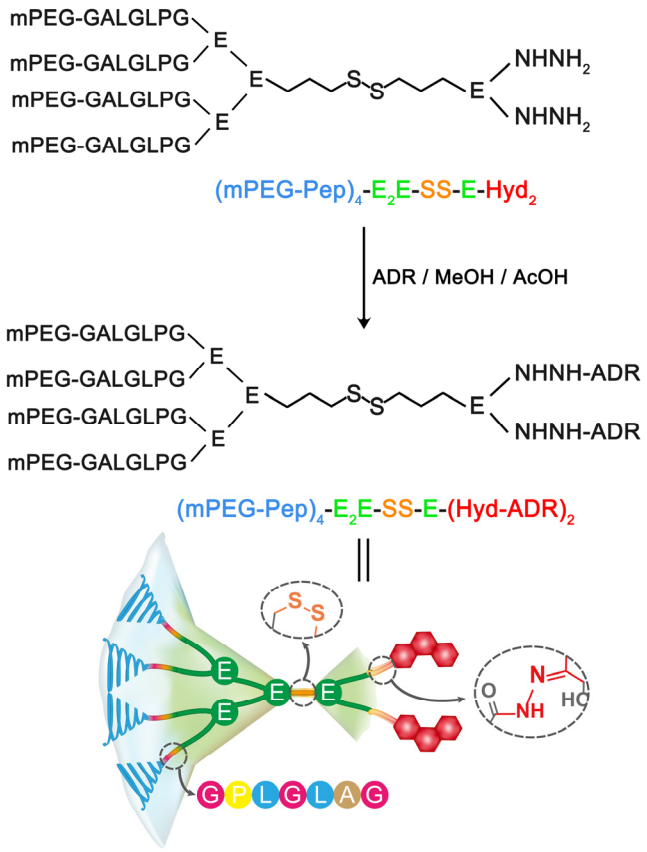
1.3.5 Synthesis of (mPEG-Pep)$_4$-E$_2$E-SS-E-Hyd$_2$



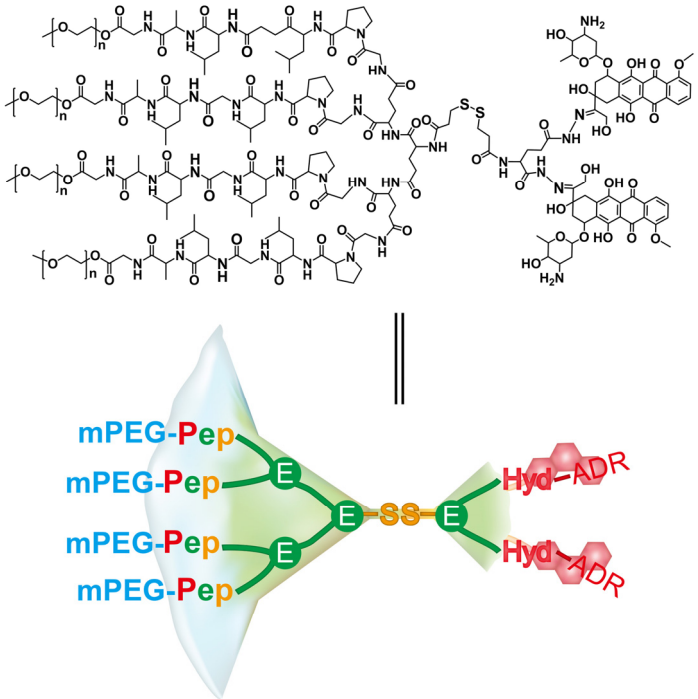
Scheme S6. Synthetic route of (mPEG-Pep)$_4$-E$_2$E-SS-E-Hyd$_2$.

(mPEG-Pep)$_4$-E$_2$E-H (1.00 g), HOOC-(CH$_2$)$_2$-SS-(CH$_2$)$_2$-E-(Hyd-Boc)$_2$ (153.14 mg, 0.27 mmol), PyBOP (140.80 mg, 0.27 mmol) and HOBT (36.50 mg, 0.27 mmol) were dissolved in anhydrous DMF and stirred at ice bath for 30 min in N$_2$ atmosphere. Then DIPEA (178 μL, 1.08 mmol) was added and the mixture was stirred for 24 h at room temperature. After removal of DMF, the product was purified by washing and silica column to get a white solid. ^{1}H NMR (400 MHz, CDCl3): 4.30 (s, CH$_2$), 3.65 (m, br, CH$_2$CH$_2$O), 3.38 (s, OCH$_3$), 2.58-2.19 (m, CH$_2$CH$_2$SSCH$_2$CH$_2$), 1.54-1.39 (m, CH, CH$_3$). Then (mPEG-Pep)$_4$-E$_2$E-SS-E-(Hyd-Boc)$_2$ (1.00 g, 0.16 mmol)was dissolved in anhydrous DCM and treated with TFA (123 μL, 1.6 mmol) in ice-bath. The mixture was stirred for 12 h. The solution was concentrated and then precipitated in excess cold ethyl ether to obtain (mPEG-Pep)$_4$-E$_2$E-SS-E-Hyd$_2$. (yield: 59%)

1.3.6 Synthesis of (mPEG-Pep)$_4$-E$_2$E-SS-E-(Hyd-ADR)$_2$



Scheme S7. Synthetic route of (mPEG-Pep)$_4$-E$_2$E-SS-E-(Hyd-ADR)$_2$.



Scheme S8. Chemical structure of (mPEG-Pep)$_4$-E$_2$E-SS-E-(Hyd-ADR)$_2$.

(mPEG-Pep)$_4$-E$_2$E-SS-E-Hyd$_2$ (0.25 g) and ADR (57.92 mg, 0.10 mmol) were dissolved

in methanol with acetic acid as an acid catalyst. The solution was stirred for 72 h. After removal of methanol, the residue was dissolved in deionized water and dialyzed in water with a molecular weight cut off (MWCO) 2000 Da membranes for 72 h and then freeze-drying. ^{1}H NMR (400 MHz, DMSO-*d6*): 5.76 (t, O<u>CH</u>(CH_2)O), 3.33 (m, br, CH_2CH_2O), 3.17 (m, 4H), 2.73 (m, CH), 2.33 (m, CH_2), 1.23 (CH_3). MALDI-TOF-MS analysis of MALDI-TOF-MS analysis of Boc-E_2E-(Pep-mPEG)$_4$ showed a broad peak with a center of mass around 6787, corresponding to theoretical mass. And the distance between neighboring peaks was corresponding to the mass of a PEG repeat unit with 44 Da.

1.4 Preparation of AuNR@DA NHs

DA and AuNR were dissolved in DMSO and mixed sufficiently with ultrasonic. Then the mixture was dropped into water under ultrasonic to self-assemble into AuNR@DA NHs by supramolecule interaction. The AuNR@DA NHs were purified by centrifuging at 1000 rpm until AuNR aggregation was removed entirely. AuNR loading capacity was measured by UV-Vis spectrum and calculated by following equation:

$$\text{Loading capacity} = \frac{Mass\ of\ AuNRs\ in\ particles}{Mass\ of\ AuNRs@Den\ NHs}$$

AuNR loading capacity was reached to 81.52 ± 3.35%.

The fluorescence of ADR with different riot of AuNR and DA was monitored by fluorescence spectrophotometer and imaged by IVIS in microplate.

1.5 Nanostructure characterization of AuNR@DA NHs

1 mL AuNR@DA NHs solution (200 μg mL^{-1}) was prepared for size and zeta potential measurement by DLS. AuNR@DA NHs solution (500 μg mL^{-1}) was dropped to copper grid and volatilized at room temperature for TEM imaging.

1.6 *In vitro* photothermal conversation.

AuNR@DA NHs and AuNR (100 μg mL^{-1}) solution and water irradiated for 10 min with a 780 nm laser (1.9 W cm^{-2}). The temperature and photothermal images were recorded by infrared (IR) thermal imaging system (FLIR, Systems, Inc, USA).

To measure the photothermal conversion effect of AuNR and AuNR@DA NHs, 1.0 mL of AuNR and AuNR@DA NHs aqueous solution was irradiated by 785 nm laser with a power density of 1.9 W cm^{-2} for 10 min. The temperature changes were recorded by IR thermal imaging system. The photothermal conversion efficiency was calculated by the following equation:

$$\text{Photothermal conversion efficiency} = \frac{hS\Delta T_{max} - Q_{dis}}{I(1 - 10^{-A_{785}})}$$

$$\mathrm{hS} = \mathrm{mC}/\tau_s$$

In the equation, h is heat transfer coefficient, S is the surface area of the container, ΔT_{max} is the difference between the maximum temperature and room temperature, Q_{dis} is hat dissipated from light absorbed by container and solvent, I is laser power, A_{785} is absorption at 785 nm.

1.7 Stability evaluation.

AuNR@DA NHs and AuNR (AuNR : 50 μg mL^{-1}) were dissolved in water, and the size distribution and UV-vis spectrum was measured at 0 h, 1 h, 2 h, 3 h, 4 h and 5 h. The experiments were carried out in triplicate.

AuNR@DA NHs and AuNR (AuNR: 100 μg mL^{-1}) solution were prepared and stored for 0 h, 1 h, 2 h, 3 h, 4 h and 5 h, respectively. At each time point, the solutions were laser (780 nm, 1.9 W cm^{-2}) irradiated for 10 min and recorded by IR thermal imaging system. The experiments were carried out in triplicate.

1.8 *In vitro* drug and AuNR release.

AuNR release. AuNR@DA NHs (AuNR : 200 μg mL^{-1}) were prepared in centrifuge tubes and incubated at 37 ℃ in triplicate. The solution was adjusted to biomimetic condition including 2 μg mL^{-1} MMP-2, 10 mM DTT and pH 5.0 at different time points. The released AuNR would precipitate with 1000 rpm centrifugation and quantified by a microplate reader.

ADR release. The *in vitro* ADR release was carried out in centrifuge tubes containing 25 mL PBS. The dialysis tubes (MWCO, 1000 Da) containing 1 mL AuNR@DA NHs solution were immersed in the centrifuge tubes. MMP-2, DTT and HCl was added to the centrifuge tubes at predesigned time to mimic the conditions corresponding to the *in vivo* delivery. 1.0 mL external medium was collected as sample and replaced by the same amount of fresh medium. The concentration of ADR was measured by fluorescence spectrum. The experiments were carried out in triplicate.

1.9 Cell uptake of AuNR and ADR

SKOV3/ADR cells were incubated with AuNR, AuNR (+L), ADR, AuNR@DA NHs and AuNR@DA NHs (+L) (AuNR: 50.0 μg mL^{-1}, ADR: 2.0 μg mL^{-1}) for 3 h. In AuNR@DA NHs (+L) group, SKOV3/ADR cells were treated with laser irradiation (780 nm, 1.9 W cm^{-2}, 10 min) after incubation with AuNR@DA NHs for 2 h, and then the cells were incubated with AuNR@DA NHs for another 1 h. For evaluating the tumor microenvironment enzyme activated cell internalization, the cells were pretreated with MMPs inhibitor (phenanthroline) and then

incubated with AuNR@DA NHs for 3 h. The cells were imaged by 2PLSM with 785 nm excitation for AuNR imaging. ADR fluorescence was excited by single-photon laser at 488 nm. Cell nucleus was stained with Hoechst 33342.

The intercellular AuNR in each group were quantified by UV-vis spectrum. The cells seed in 6 well plate and treated as above. The cells were collected by centrifugation and lysed by RIPA lysis buffer. The protein concentration was measured by Pierce™ BCA Protein Assay Kit (ThermoFisher Scientific, USA). AuNR content in each well were determined by measuring the UV-vis absorbance at 785 nm using a microplate reader. ADR was quantified by flow cytometry (Beckman Coulter, USA).

1.10 Intracellular delivery

SKOV3/ADR cells were pretreated with BSO (0.1 mM, 12 h) to downregulate the intracellular GSH level. Then the cells incubated with AuNR@DA NHs for 4 h to evaluate the GSH dependent AuNR release.

SKOV3/ADR cells treated with AuNR@DA NHs for 4 h, and then the medium was replaced with fresh culture medium. The cells were laser irradiated (785 nm, 1.9 W cm^{-2}, 10 min) and culture in culture medium containing AuNR@DA NHs for 2 h. The cells were washed with PBS and stained by Lysotracker Blue (Life, USA).

For evaluating the nucleus delivery of ADR, SKOV3/ADR cells were incubated with ADR, AuNR@DA NHs and AuNR@DA NHs (+L, 12 h post-incubation) for 24 h. The cells were washed with PBS and cell nucleus were stained with Hoechst 33342.

1.11 Cell viability assay

SKOV3/ADR cells were seeded in 96 well plate (8000 cells per well) and cultured for 24 h. To determine the appropriate ratio of AuNR and ADR, the cells were treated with AuNR@DA NHs with different AuNR content for 24 h, and ADR was fixed at 1.0 μg mL^{-1}. After the medium w ere removed and replaced by fresh culture medium at 12 h, the cells were laser irradiated, and then the cells cultured in culture medium containing different formulations for 12 h. To evaluate the *in vitro* antitumor efficiency of AuNR@DA NHs, the cells were treated with AuNR (+L), free ADR, AuNR@DA NHs (AuNR: ADR = 25: 1) and AuNR@DA NHs (+L, 24 h post-incubation) for 48 h. The cell viability was measured by CCK-8 assay. Laser irradiation condition was 1.9 W cm^{-2} for 10 min using 785 nm laser.

1.12 Mitochondrial membrane potential measurement

SKOV3/ADR cells were seeded in confocal dishes and cultured for 24 h. The cells were treated with AuNR (+L, 10 h post-incubation), free ADR, AuNR@DA NHs and AuNR@DA

NHs (+L, 10 h post-incubation) (50.0 μg mL^{-1} AuNR, 2.0 μg mL^{-1} ADR) for 12 h. Then the cells were stained with MitoView 633 and imaged by CLSM. Relative mitochondrial membrane potential was quantified by fluorescence intensity analysis.

1.13 Intracellular ROS detection

SKOV3/ADR cells (about 2×10^4 cells) were seeded in 24-well plate and culture for 24 h. Then the cells were treated with AuNR (+L, 10 h post-incubation), free ADR, AuNR@DA NHs and AuNR@DA NHs (+L, 10 h post-incubation) (50.0 μg mL^{-1} AuNR, 2.0 μg mL^{-1} ADR) for 12 h. The cells were stained with DCF-DA to detect the intracellular ROS and imaged by inverted fluorescence microscope (Leica, Germany). The cell nucleus was stained with Hoechst 33342.

1.14 Caspase-3 activity assay

SKOV3/ADR cells (about 5×10^4 cells) were seeded in 12-well plate and culture for 24 h. The cells were treated with AuNR (+L, 10 h post-incubation), free ADR, AuNR@DA NHs and AuNR@DA NHs (+L, 24 h post-incubation) (25.0 μg mL^{-1} AuNR, 1.0 μg mL^{-1} ADR) for 48 h. The cells (about 3×10^3 cells) were seed in confocal dishes and treated as above. Then cells were stained with CellEvent™ Caspase-3/7 red detection reagent and imaged by CLSM.

1.15 Cell apoptosis and necrosis analysis

SKOV3/ADR cells (about 8×10^4 cells) were cultured in 6-well plate for 24 h. The cells were incubated with AuNR (+L, 24 h post-incubation), free ADR, AuNR@DA NHs and AuNR@DA NHs (+L, 24 h post-incubation) for 48 h (50.0 μg mL-1 AuNR, 2.0 μg mL-1 ADR). The cells were digested by Trypsin-EDTA and collected by centrifugation. Then the cells were stained with 7-AAD and Annexin V-APC and analyzed by flow cytometry. The data were analyzed by FlowJo software.

The cells were treated with AuNR@DA NHs (+L, 24 h post-incubation) for 48 h. The cells were digested by Trypsin-EDTA and collected by centrifugation. Afterwards, the cells were fixed by 0.5% glutaraldehyde PBS solution for 30 min and then centrifuged at 10000 rpm for 15 min. Secondary fixation was carried out in 3% glutaraldehyde PBS solution and stored at 4 ℃. The cells were sliced and imaged by TEM.

1.16 Photothermal imaging of SKOV3/ADR cells

SKOV3/ADR cells were cultured in 24-well plate for 24 h and incubated with AuNR and AuNR@DA NHs for 24 h. The cells were washed with PBS three times and digested with trypsin. Then the cells were collected into centrifuge tube and irradiated by NIR laser. Photothermal images and temperature were recorded by near infrared imaging system.

1.17 Live/dead cell stain

SKOV3/ADR cells (about 2×10^4 cells) were cultured in 24-well plate for 24 h. The cells were treated with AuNR (+L, 24 h post-incubation), free ADR, AuNR@DA NHs and AuNR@DA NHs (+L, 24 h post-incubation) (50.0 μg mL^{-1} AuNR, 2.0 μg mL^{-1} ADR) for 48 h. Then the cells were stained with FDA (live cells, 10 μg mL^{-1}) and PI (dead cells, 5 μg mL^{-1}) for 15 min. The cells were imaged by an inverted fluorescence microscope.

1.18 *In vitro* tumor penetration investigation

In vitro tumor penetration of AuNR and ADR was investigated on multicellular spheroids (MTSs). SKOV3/ADR MTSs were formed in 1% agarose coated 6-well plate with 3000 cells per well. The MTSs were cultured for about 10 days and reached to ~ 500 μm in diameter. The MTSs incubated with AuNR (+L, 4 h post-incubation), free ADR, AuNR@DA NHs and AuNR@DA NHs (+L, 4 h post-incubation) (50.0 μg mL^{-1} AuNR, 2.0 μg mL^{-1} ADR) for 6 h. The MTSs were washed by PBS and imaged with 2PLSM (5 μm per section). Surface plots of images were analyzed by ImageJ software.

1.19 *In vitro* antitumor effect on SKOV3/ADR MTSs

SKOV3/ADR MTSs were treated with AuNR (+L, 12 h post-incubation), free ADR, AuNR@DA NHs and AuNR@DA NHs (+L, 12 h post-incubation) (50.0 μg mL^{-1} AuNR, 2.0 μg mL^{-1} ADR) for 24 h. Laser irradiation was carried out at 12 h. Then the MTSs were stained with FDA/PI for 30 min. The MTSs were imaged by CLSM. The percentage of dead cells (PI positive ratio) was analyzed by LAS X software.

The morphology of treated MTSs were observed by scanning electron microscope (SEM). SKOV3/ADR MTSs were transferred into confocal dishes and culture 6 h for adherence. Then the MTSs were treated as above. The MTSs were fixed with 4% paraformaldehyde for 2 h and washed with PBS and H_2O. MTSs were dehydrated in an ethanol gradient including 10%, 30%, 50%, 70%, 100%, 50% (ethanol: hexamethyldisilazane (HMDS)) and 100% HMDS with 10 min duration. The MTSs were dried overnight and coated with gold for SEM imaging.

To evaluate the antitumor efficacy of AuNR@DA NHs, SKOV3/ADR MTSs were treated with AuNR (+L, 12 h post-incubation), free ADR, AuNR@DA NHs and AuNR@DA NHs (+L, 12 h post-incubation) (50.0 μg mL^{-1} AuNR, 2.0 μg mL^{-1} ADR) for 24 h. The culture medium was replaced with fresh culture medium and cultured for 7 d. The MTSs were imaged every day to record the volume. The diameter of MTSs was measured by ImageJ software. The volume of MTSs was calculated by following formulation: $V = 4\pi R^3/3$. 15 MTSs were calculated in each group. MTSs inhibition ratio was calculated by the following formula: $\text{inhibition ratio (\%)} = \frac{V_{control}-V_A}{V_{control}} \times 100\%$, where $V_{control}$ is average MTS volume of control

group, V_A is that of treated group.

1.20 *In vivo* photothermal imaging (PTI)

Animals and tumor xenograft models. All animal experiments were approved by the ethics committee of Sichuan University. 3×10^6 SKOV3/ADR cells suspended in 100 μL PBS were subcutaneously injected onto the back of BALB/c nude mice. The mice were administrated until the tumor volume reached to 100 mm^3.

In vivo PTI. SKOV3/ADR tumor-bearing mice were i.v. injected with saline, AuNR and AuNR@DA NHs (50.0 mg AuNR kg^{-1}, 2.0 mg ADR kg^{-1}). After 6 h post-injection, the tumor was irradiated for 10 min with a 785 nm laser at 1.9 W cm^{-2}. The photothermal images were captured with an interval of 0.5 min.

1.21 *In vivo* tumor penetration and drug distribution

In vivo PA imaging. SKOV3/ADR tumor-bearing mice were administrated with AuNR and AuNR@DA NHs (50.0 mg AuNR kg^{-1}, 2.0 mg ADR kg^{-1}) with and without laser irradiation (785 nm, 1.9 W cm^{-2}, 6 h post-injection). Laser irradiation was conducted for 3 times with 2 min each time and the time interval was 5 min. The temperature of AuNR@DA NHs group increased to ~50℃. The temperature was monitored by thermal imaging system. The mice anesthetized with 3% isoflurane, and then ultrasound gel was smeared on the skin. The mice were imaged by photoacoustic imaging system with a ~0.3 mm step distance, and the maximum contrast PA signal was obtained at 780 nm.

In vivo fluorescence imaging. The fluorescence drug ADR was used for *in vivo* fluorescence imaging. Tumor-bearing mice were administrated with free ADR, AuNR@DA NHs and AuNR@DA NHs (+L, 6 h post-injection). Laser irradiation condition was the same as above. Then the mice were imaged by IVIS.

Tumor cryo-sections were used to evaluate the *in vivo* tumor penetration of AuNR and ADR. The tumor-bearing mice were treated with AuNR, free ADR, AuNR (+L, 6 h post-injection), AuNR@DA NHs and AuNR@DA (+L, 6 h post-injection) (50.0 mg AuNR kg^{-1}, 2.0 mg ADR kg^{-1}) as above for 24 h. The tumor tissues were dissected and sectioned. Tumor vessels were stained with CD-31 antibody, and cell nucleus was stained with Hoechst 33342. The sections were imaged by 2PLSM.

1.22 Blood perfusion assay

SKOV3/ADR tumor-bearing mice were injected with saline, free ADR, AuNR and AuNR@DA NHs (50.0 mg AuNR kg^{-1}, 2.0 mg ADR kg^{-1}). The mice were irradiated at 6 h post-injection using NIR laser at 785 nm with a power density of 1.9 W cm^{-2}. Laser irradiation

condition was the same as above. After laser irradiation, the blood flow was measured by moor FLPI-2 full-field laser perfusion imager and analyzed by moorFLPI software.

1.23 H&E and immunohistochemical analysis

SKOV3/ADR tumor-bearing mice were treated with AuNR, free ADR, AuNR (+L, 6 h post-injection), AuNR@DA NHs and AuNR@DA (+L, 6 h post-injection) (50.0 mg AuNR kg^{-1}, 2.0 mg ADR kg^{-1}). Laser irradiation was conducted for 3 times with 2 min each time and the time interval was 5 min. The tumor tissues were dissected at 3 d post injection and sectioned for H&E and immunohistochemical analysis. The sections stained with H&E for pathology analysis. Apoptosis and necrosis in tumor were analyzed by Tunel and PARP immunofluorescence assay.

1.24 *In vivo* distribution of ADR and tumor accumulation of AuNR

SKOV3/ADR tumor-bearing mice were treated with AuNR, free ADR, AuNR (+L, 6 h post-injection), AuNR@DA NHs and AuNR@DA (+L, 6 h post-injection) (50.0 mg AuNR kg^{-1}, 2.0 mg ADR kg^{-1}). Laser irradiation was conducted for 3 times with 2 min each time and the time interval was 5 min. The mice were sacrificed and the tissues were collected after 24 h post injection. Tissues of 0.1 mg were homogenized in KH_2PO_4 (10 mL/g tissue). Chloroform and isopropanol (4/1, v/v) solution was used for ADR extraction, and then the mixture were centrifuged at 13 000 r.p.m. for 10 min. The organic phases were collected for ADR content analyzed using HPLC. For Au content analysis, tumor tissues were digested in HNO_3 and H_2O_2 (4: 3, v/v) solution at 150 ℃ overnight. When the residual decreased to about 1.0 mL, aqua regia was added to 4.0 mL until the solution became clear and the volume decreased to about 0.5 mL. After the residual solution was cooled down, 2.0 mL dd H_2O was added for Au content analysis by AAS. The content of ADR and Au was expressed as the percentage of injected dose per tissue weight (% ID g^{-1} or % ID). The tissue distribution of AuNR/ADR was calculated as following formula:

$$\text{Tissue distribution (\%ID)} = \frac{M_{tissue}}{M_{injection}} \times 100\%$$

$$\text{Tissue distribution (\%ID/g)} = \frac{M_{tissue}}{M_{injection}} \times 100\%/m$$

M_{tissue}, the amount of AuNR/ADR in tissues; $M_{injection}$, injection dose of AuNR/ADR; m, weight of tissues.

2. Results

2.1 Characterizations of denermiers

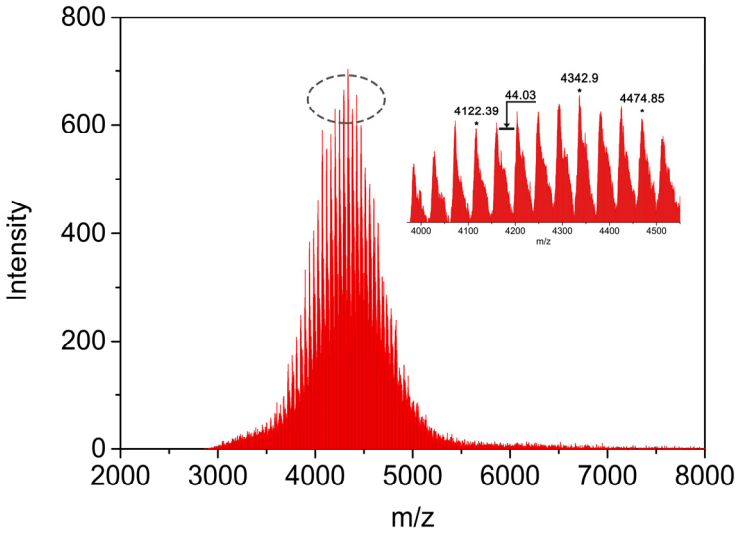


Fig. S1 MALDI-TOF mass spectrum of (mPEG-Pep)$_4$-E$_2$E-Boc.

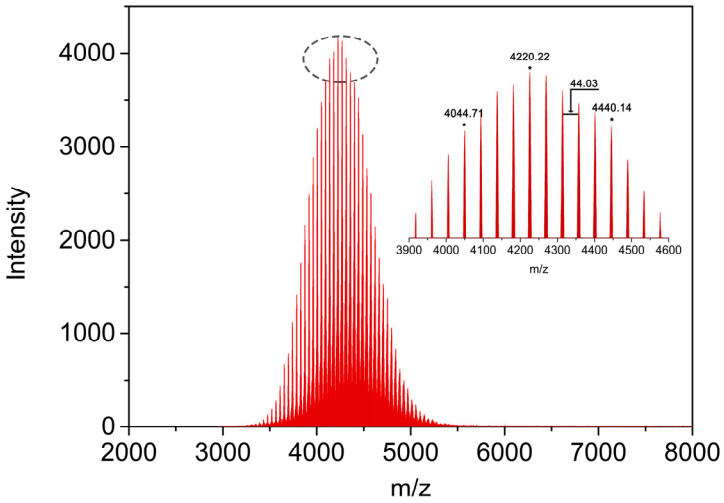


Fig. S2 MALDI-TOF mass spectrum of (mPEG-Pep)4-E$_2$E-H.

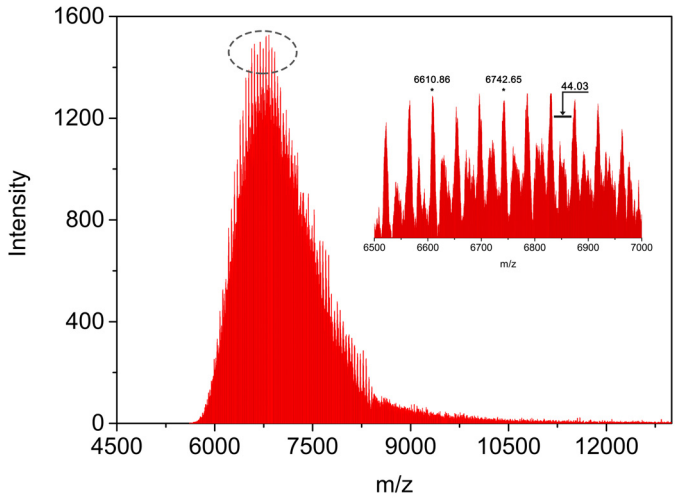


Fig. S3 MALDI-TOF mass spectrum of (mPEG-Pep)4-E_2E-SS-E-(Hyd-ADR)$_2$.

2.2 TEM image of AuNR

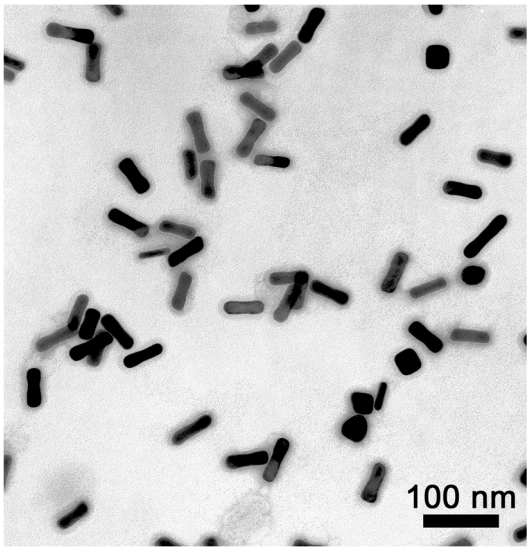


Fig. S4. TEM image of AuNR.

2.3 *In vitro* photothermal conversion

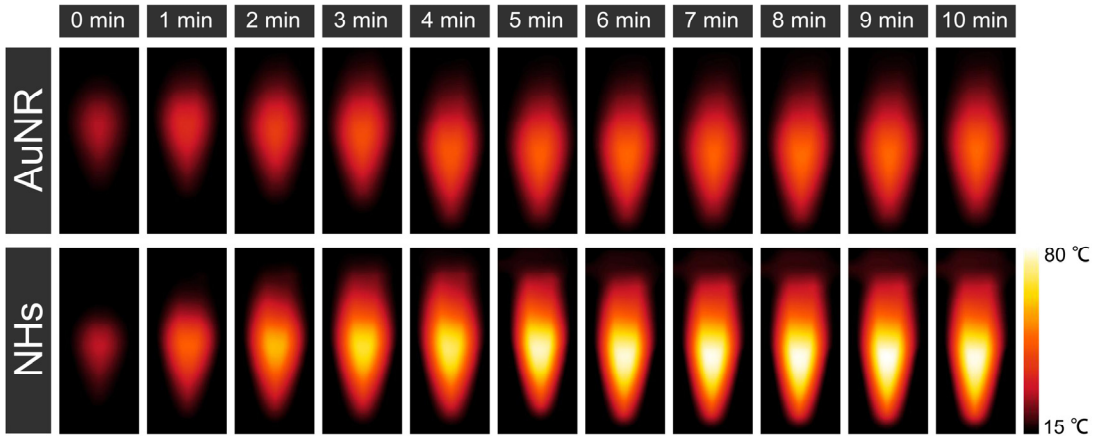


Fig. S5 Photothermal images of AuNR and AuNR@DA NHs (AuNR: 100 μg mL^{-1}). Laser irradiation: 785 nm, 1.9 W cm^{-2}, 10 min.

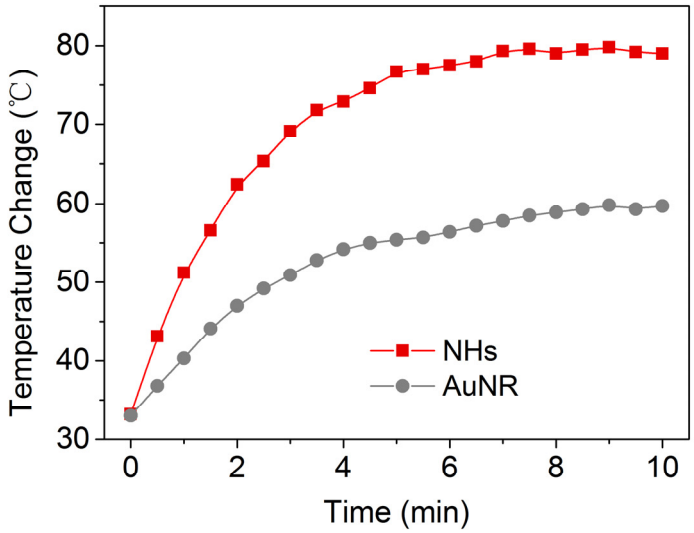


Fig. S6 Photothermal curves of AuNR and AuNR@DA NHs (AuNR: 200 μg mL^{-1}). Laser irradiation: 785 nm, 1.9 W cm^{-2}, 10 min.

2.4 AFM image of AuNR@DA NHs

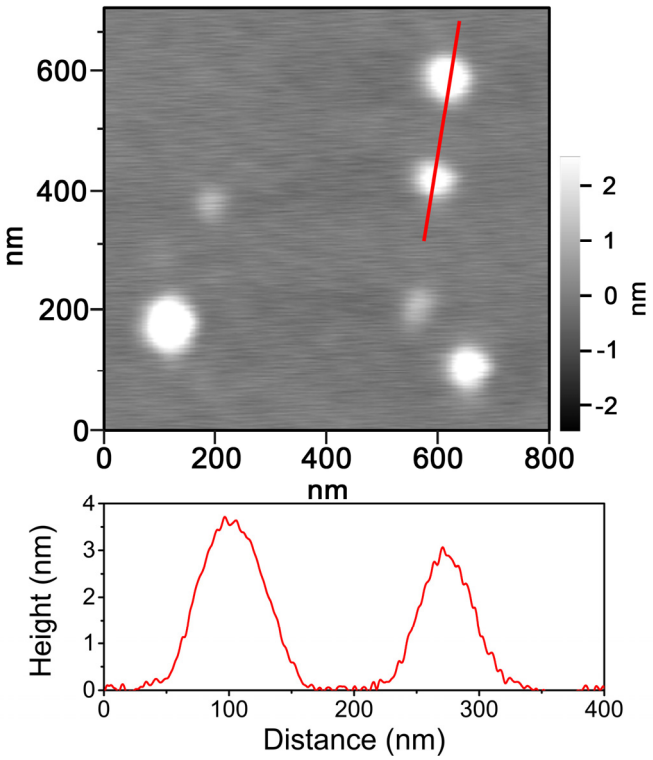


Fig. S7 AFM image of AuNR@DA NHs and morphology profile along the red line.

2.5 TEM images of AuNR@DA NHs

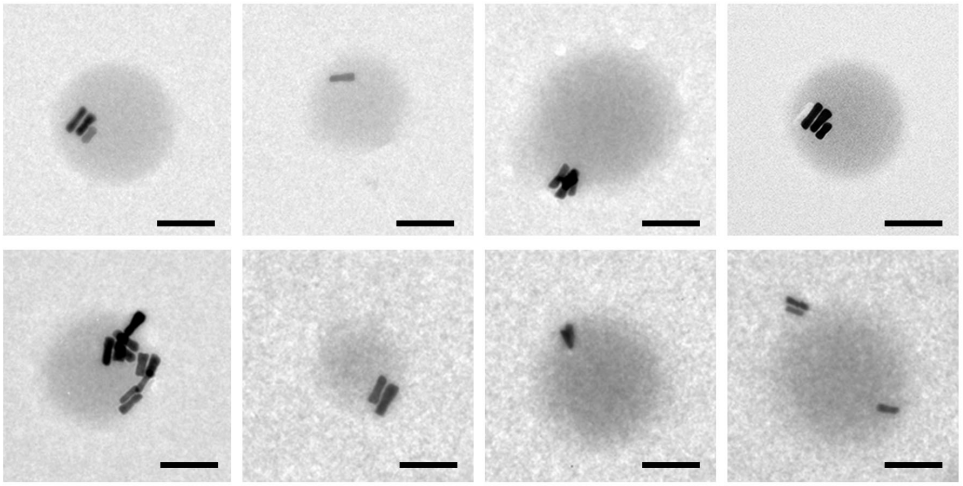


Fig. S8 TEM images of AuNR@DA NHs. (scale bar: 100 nm)

2.6 Zeta potential of AuNR@DA NHs

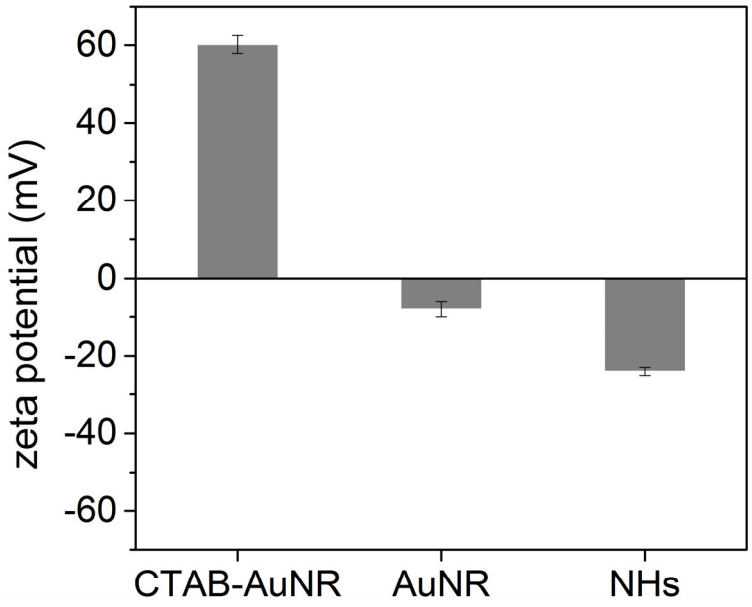


Fig. S9 Zeta potential of CTAB-AuNR, AuNR and AuNR@DA NHs.

2.7 Fluorescence quenching of ADR

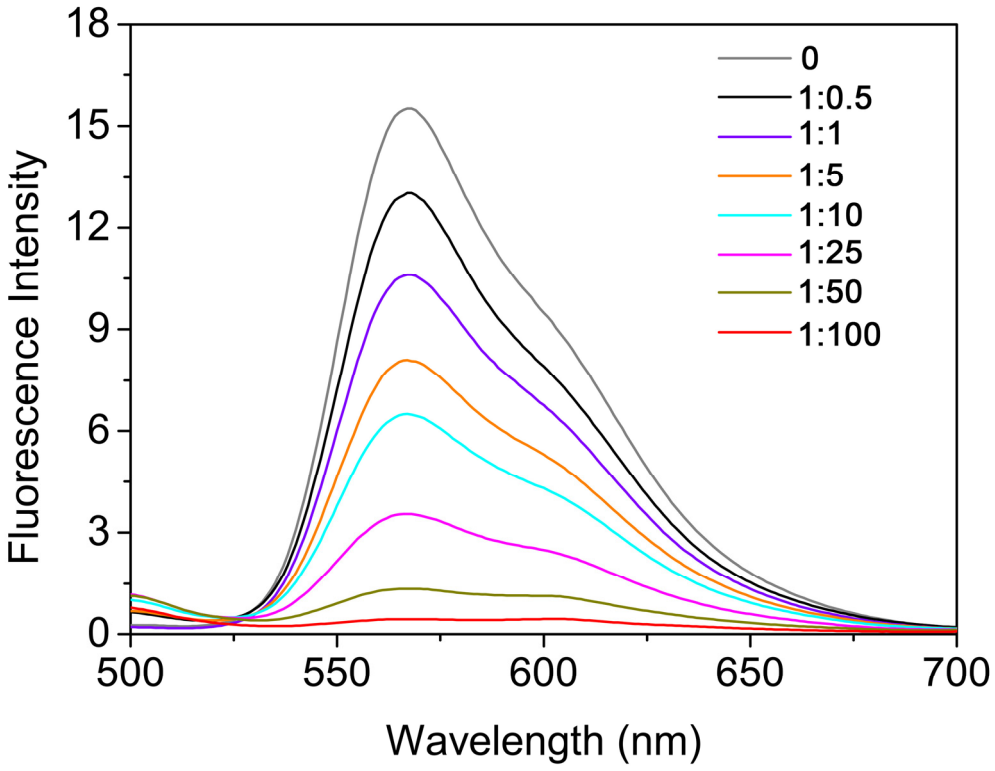


Fig. S10 Fluorescence spectrum of ADR with different ratio of DA and AuNR in AuNR@DA NHs. (λ_{ex} = 488 nm)

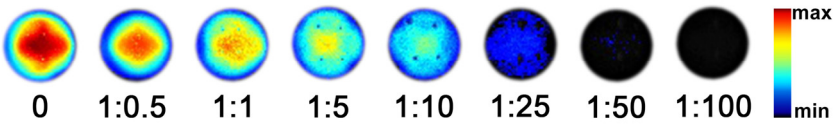


Fig. S11 Fluorescence images of ADR with different ratio of ADR and AuNR in AuNR@DA NHs.

2.8 *In vitro* antitumor effect of AuNR@DA NHs

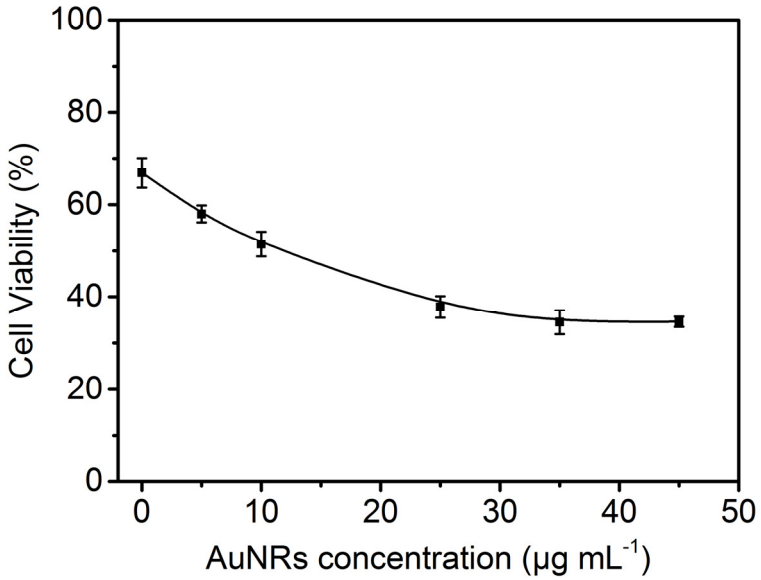


Fig. S12 Cell viability of SKOV3/ADR cells which treatment with different ratio of AuNR and DA. ADR concentration of AuNR@DA NHs was 1.0 μg mL^{-1}. AuNR concentration was changed from 0 to 60 μg mL^{-1}. The result indicated that the optimal ratio of AuNR and ADR was 25: 1.

2.9 *In vitro* stability of AuNR@DA NHs

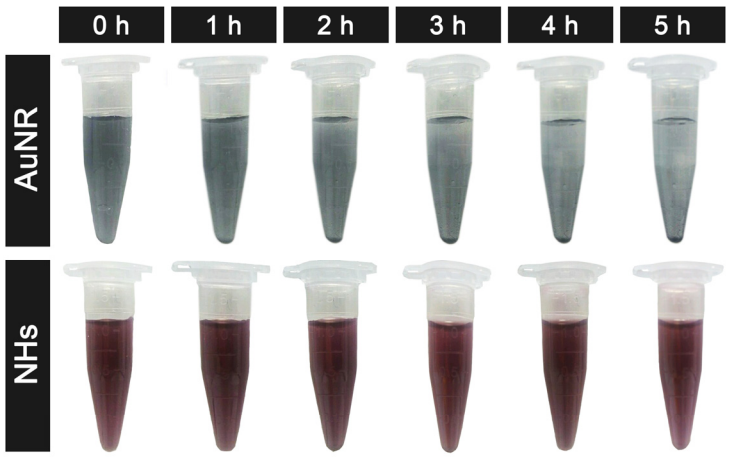


Fig. S13 Optical images of AuNR and AuNR@DA NHs solutions. The photographs of AuNR and AuNR@DA NHs solutions were imaged at different storage period.

2.10 Photothermal effect

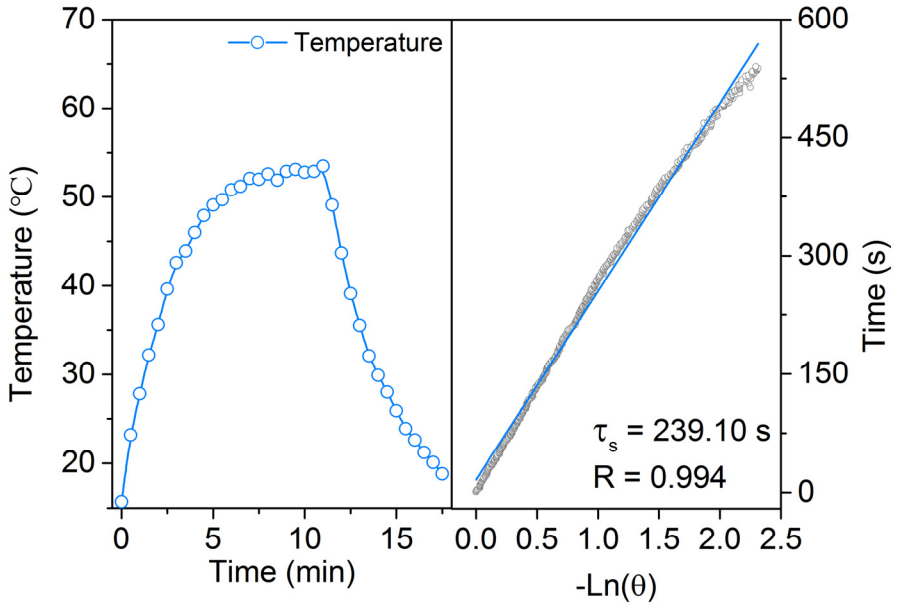


Fig. S14 Photothermal effect of AuNR aqueous dispersion irradiated with 785 nm laser (1.9 W cm^{-2}) and shutting off the laser (left). Time constant for heat transfer from the system (τ_s = 239.10 s).

2.11 Photothermal stability in aqueous solution

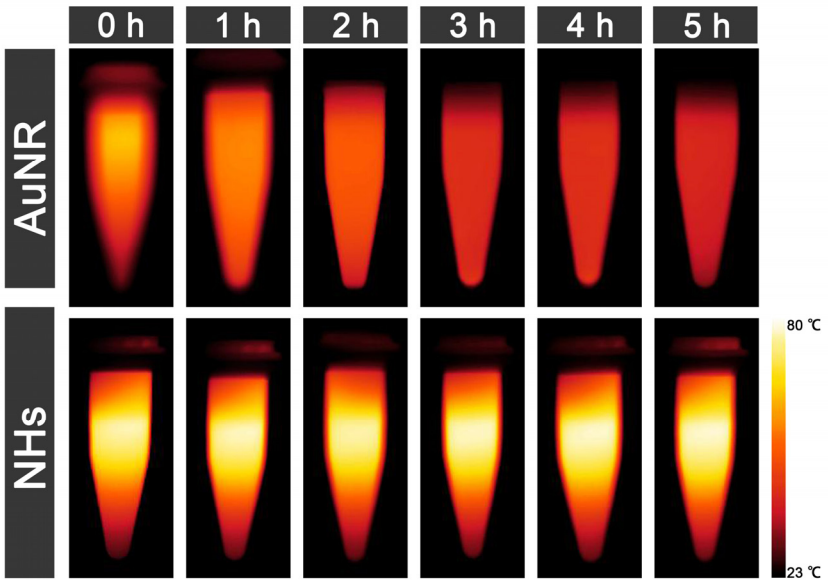


Fig. S15 Photothermal images of AuNR and AuNR@DA NHs after stored for different period. The images were captured at 10 min during laser irradiation (785 nm, 1.9 W cm^{-2}).

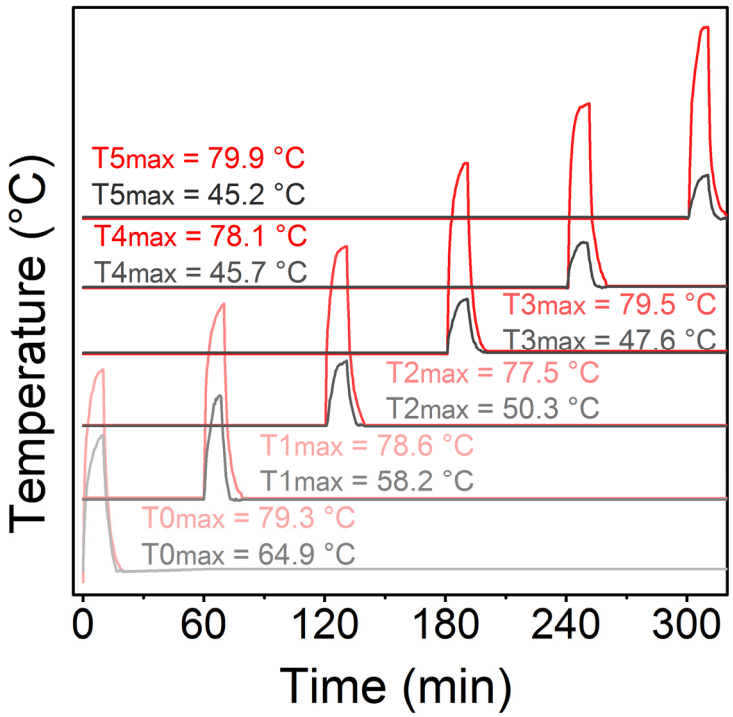


Fig. S16 Photothermal effect of AuNR and AuNR@DA NHs solution (AuNR: 300.0 μg mL^{-1}) after stored for different period. (785 nm laser, 1.9 W cm^{-2})

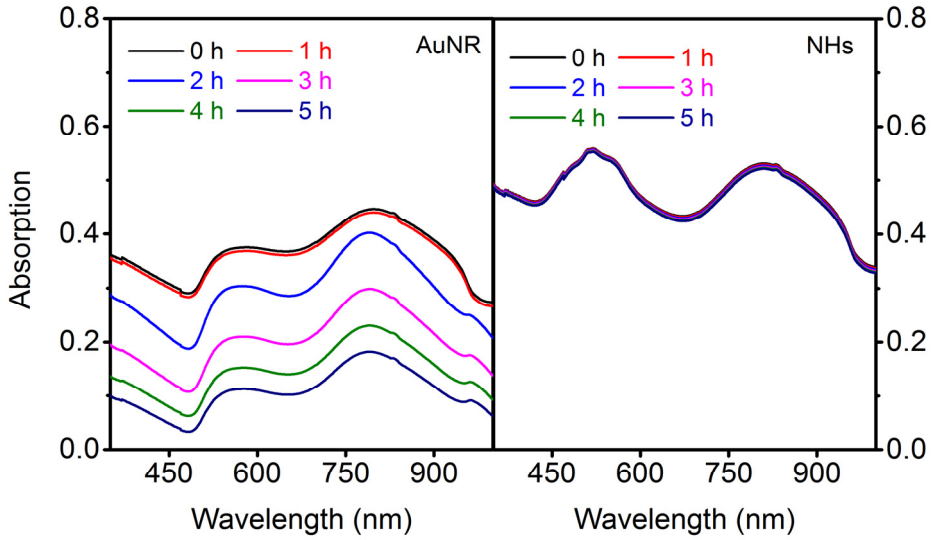


Fig. S17 UV-Vis spectrum of AuNR and AuNR@DA NHs (AuNR 200.0 μg mL^{-1}) after stored for different times.

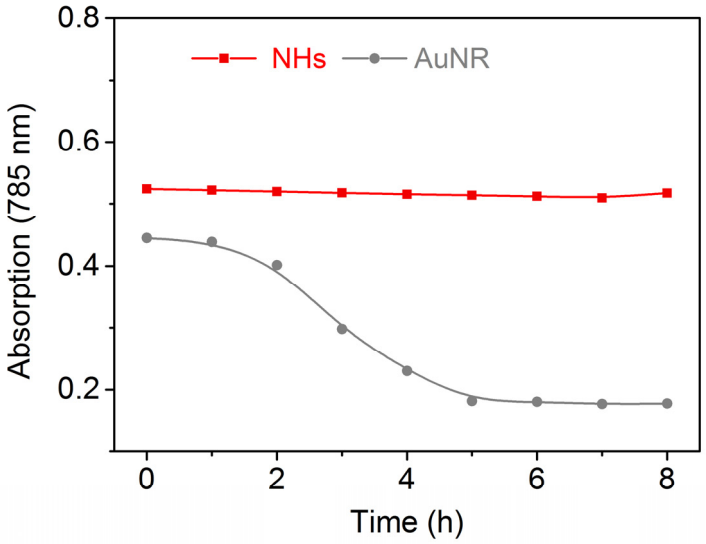


Fig. S18 Variation of the absorption at 785 nm after stored for different times.

2.12 Fluorescence changes in different stimulations

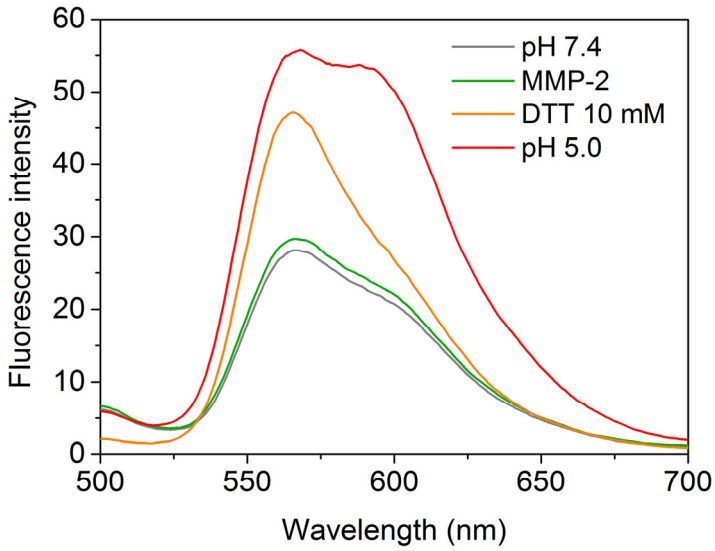


Fig. S19 Fluorescence changes of AuNR@DA NHs solution in different biomimetic buffer including pH 7.4, 2.0 μg mL^{-1} MMP-2, 10 mM DTT and pH 5.0.

2.13 *In vitro* AuNR and ADR release

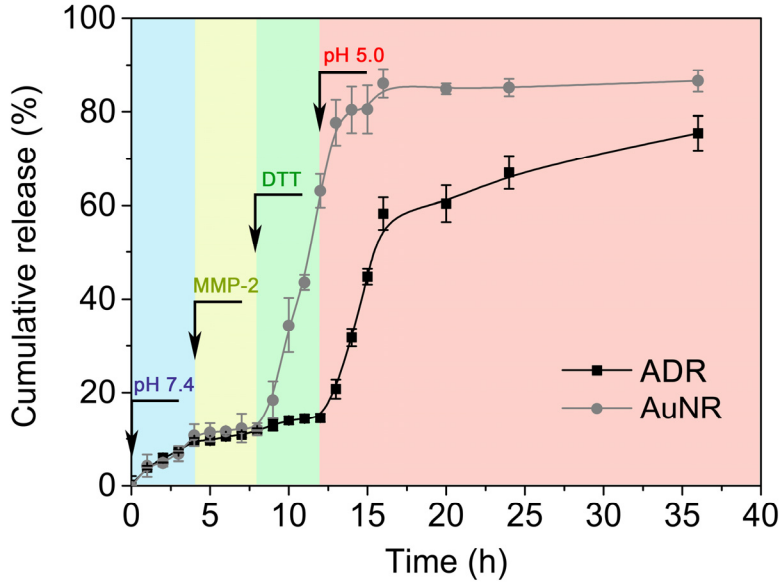


Fig. S20 *In vitro* release of AuNR and ADR with different stimulations including 2 μg mL^{-1} MMP-2, 10 mM DTT and pH 5.0. (mean ± SD, n = 3)

2.14 Protein adsorption of AuNR and AuNR@DA NHs

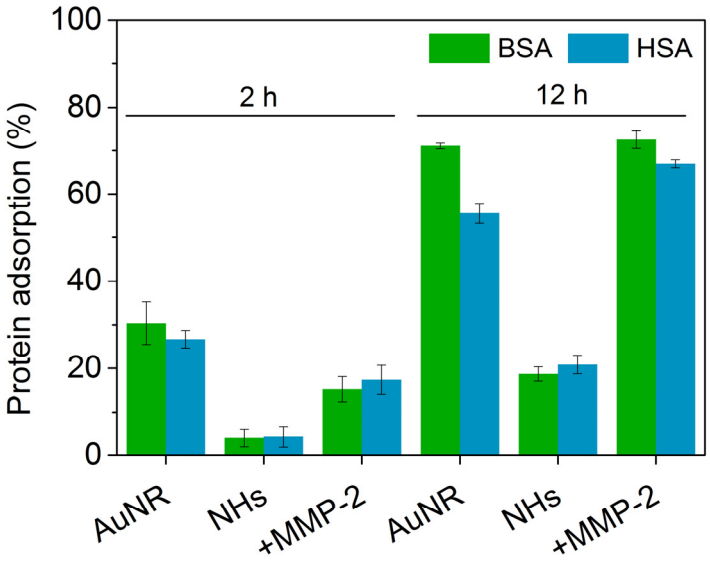


Fig. S21 BSA and HSA adsorption on AuNR and AuNR@DA NHs after 2 h and 12 h incubation at 37 °C. (mean ± SD, n = 3)

2.15 Cell uptake of AuNR and ADR

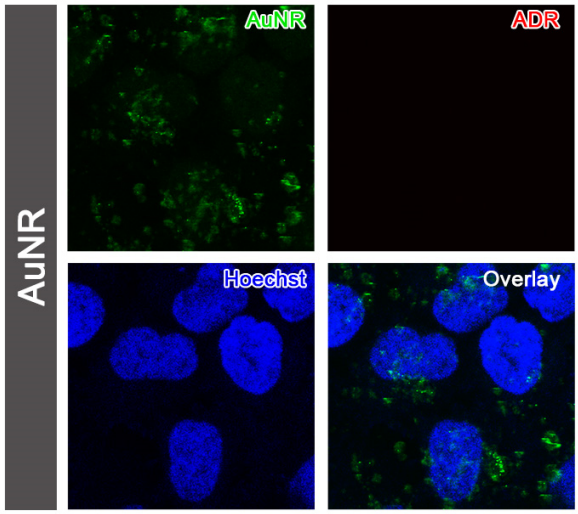


Fig. S22 CLSM images of SKOV3/ADR cells incubate with AuNR for 3 h.

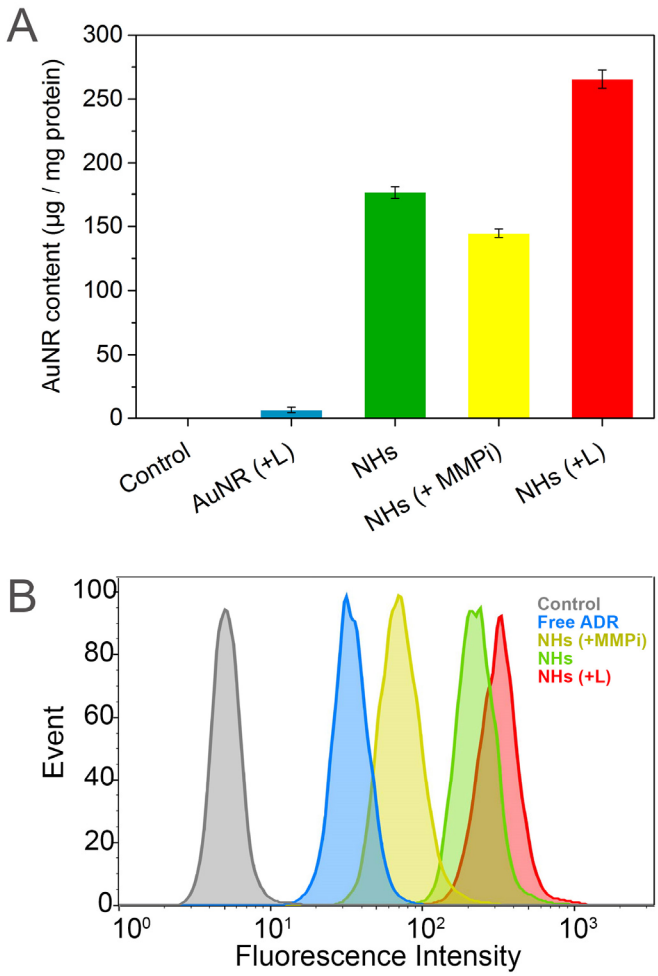


Fig. S23 Quantitative analysis of intracellular AuNR and ADR. SKOV3/ADR cells were treated with AuNR, AuNR (+L, 2 h post-incubation), AuNR@DA NHs, AuNR@DA NHs (MMP_i: MMPs inhibitor pretreated) and AuNR@DA NHs (+L, 2 h post-incubation) (50.0 μg mL^{-1} AuNR, 2.0 μg mL^{-1} ADR) for 3 h.

2.16 Intracellular GSH activated AuNR@DA NHs disintegration and AuNR release

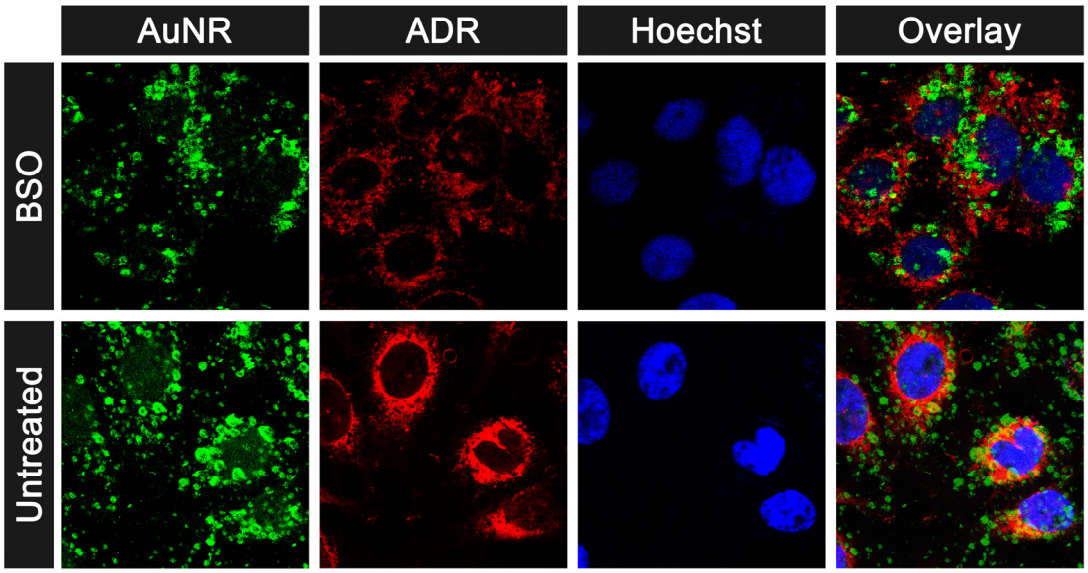


Fig. S24 2PLSM images of SKOV3/ADR cells with and without BSO pretreated. SKOV3/ADR cells were incubated with AuNR@DA NHs for 4 h. Green: AuNR; Red: ADR; Blue: cell nucleus.

2.17 NIR laser irradiation induced endosomal escape

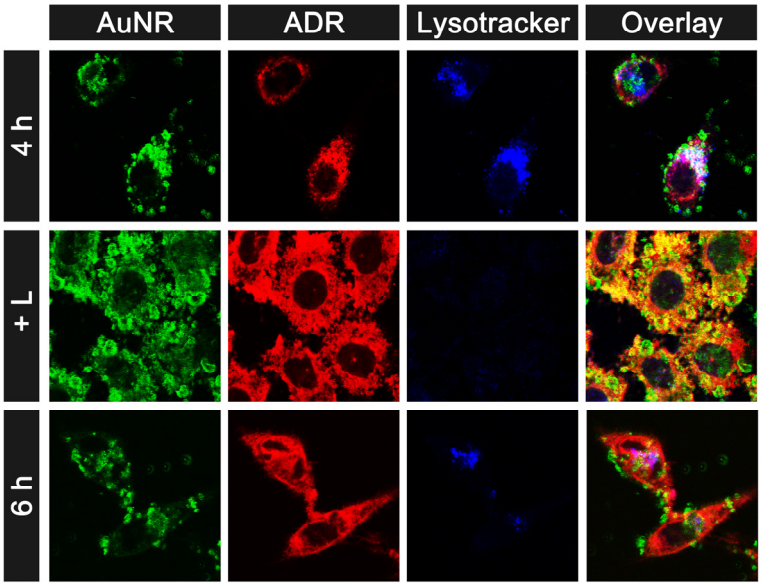


Fig. S25 2PLSM images of SKOV3/ADR cells treated with AuNR@DA NHs for different times and AuNR@DA NHs (+L, 4 h post-incubation) for 6 h. Green: AuNR; Red: ADR; Blue: lysosome.

2.18 Hyperthermia induced mitochondrial membrane potential depletion

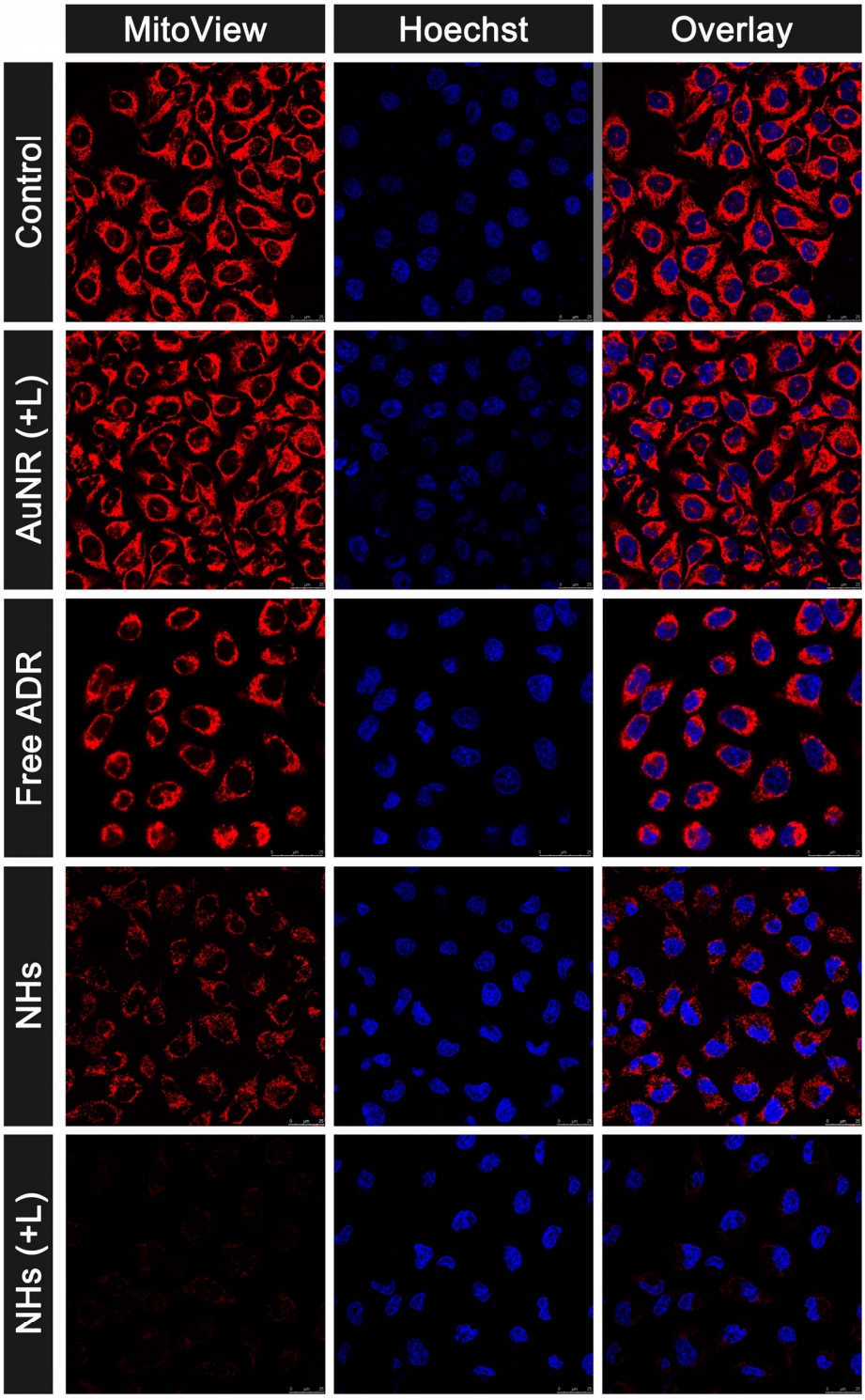


Fig. S26 CLSM images of SKOV3/ADR cells treated with AuNR (+L, 10 h post-incubation), free ADR, AuNR@DA NHs and AuNR@DA NHs (+L, 10 h post-incubation) (AuNR: 50.0 μg mL^{-1}, ADR: 2.0 μg mL^{-1}) for 12 h and stained with MitoView 633. MitoView (red fluorescence) was used to indicate mitochondrial membrane potential. The fluorescence intensity is positive correlation with mitochondrial membrane potential. Cell nucleus were stained with Hoechst 33342 (blue fluorescence).

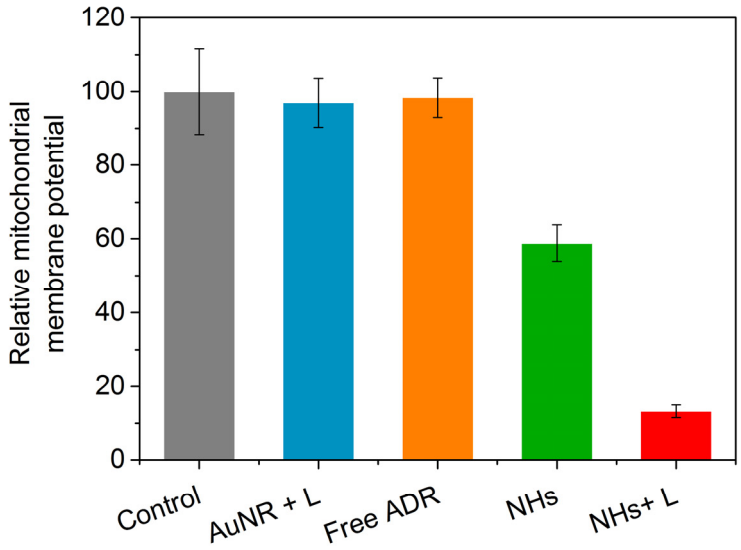


Fig. S 27 Mitochondrial membrane potential of SKOV3/ADR cells treatment with AuNR (+L, 10 h post-incubation), free ADR, AuNR@DA NHs and AuNR@DA NHs (+L, 10 h post-incubation) (AuNR: 25.0 μg mL^{-1}, ADR: 1.0 μg mL^{-1}) for 12 h. The relative mitochondrial membrane potential was quantified by the mean fluorescence intensity of MitoView 633 peer cell. (mean ± SD, n = 50)

2.19 Intracellular ROS level

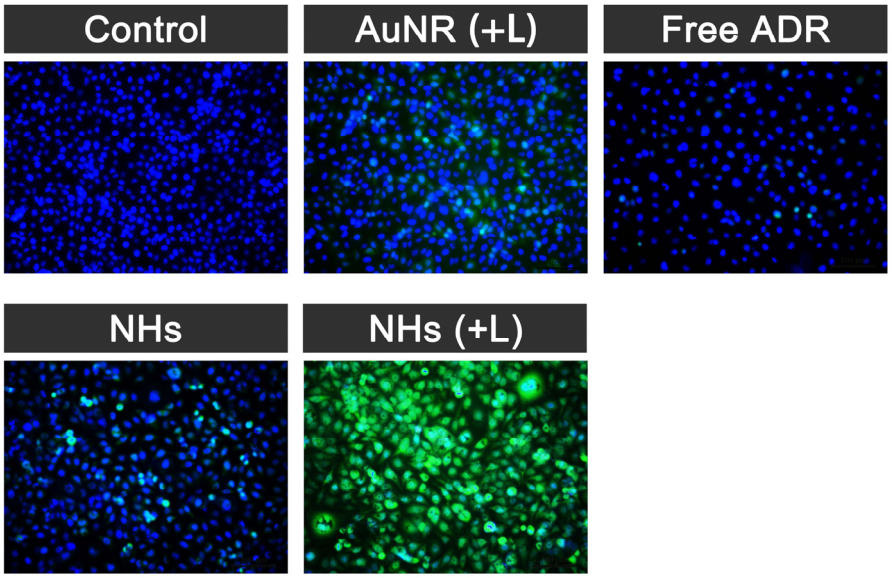


Fig. S28 Intracellular ROS level of SKOV3/ADR cells treatment with AuNR (+L, 10 h post-incubation), free ADR, AuNR@DA NHs and AuNR@DA NHs (+L, 10 h post-incubation) (AuNR: 50.0 μg mL^{-1}, ADR: 2.0 μg mL^{-1}) for 12 h. DCF-DA was used as intracellular ROS indicator.

2.20 Activated caspase-3 in SKOV3/ADR cells

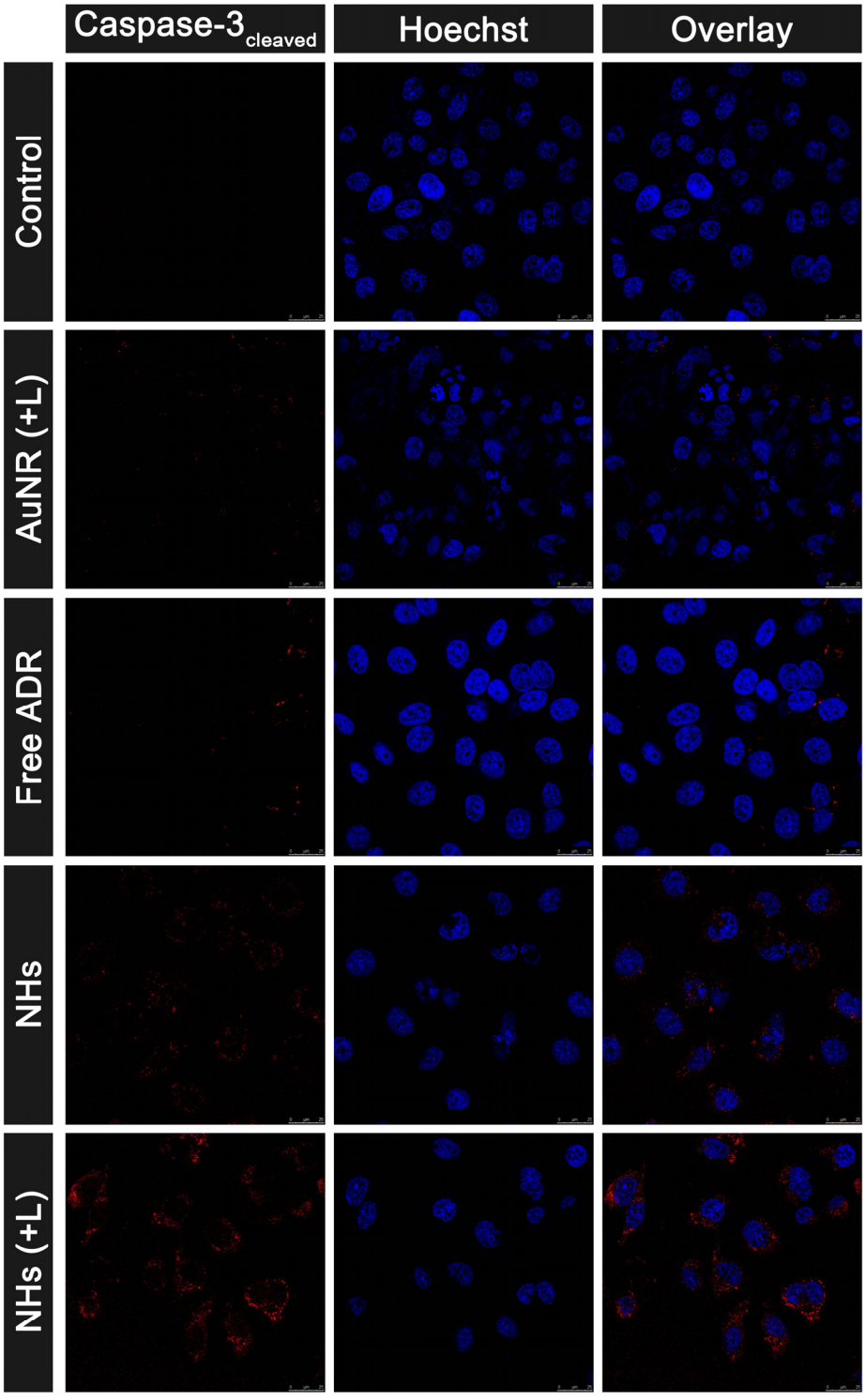


Fig. S29 Caspase-3 activity in SKOV3/ADR cells treatment with AuNR (+L, 24 h post-incubation), free ADR, AuNR@DA NHs and AuNR@DA NHs (+L, 24 h post-incubation) (AuNR: 25.0 μg mL^{-1}, ADR: 1.0 μg mL^{-1}) for 48 h. CellEvent Caspase-3 red detection reagent was used to detect activated caspase-3. The fluorescence intensity is positive correlation with caspase-3 activity. Cell nucleus was stained with Hoechst 33342 (blue fluorescence).

2.21 In vitro photothermal imaging and Live/dead cell assay

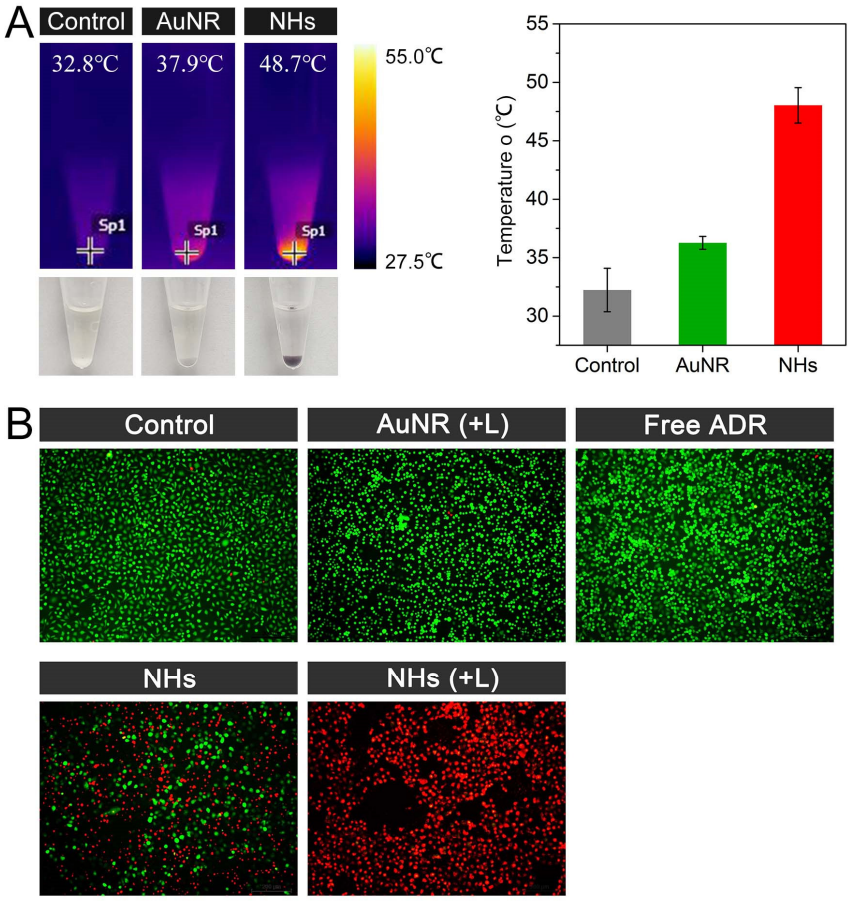


Fig. S30 (A) Optical images of AuNR and AuNR@DA NHs solutions. (B) Fluorescence images of FDA/PI stained SKOV3/ADR cells. SKOV3/ADR cells were incubated with AuNR (+L, 24 h post-incubation), free ADR, AuNR@DA NHs and AuNR@DA NHs (+L, 24 h post-incubation) (AuNR: 50.0 μg mL^{-1}, ADR: 2.0 μg mL^{-1}) for 48 h.

2.22 *In vitro* tumor penetration of AuNR@DA NHs

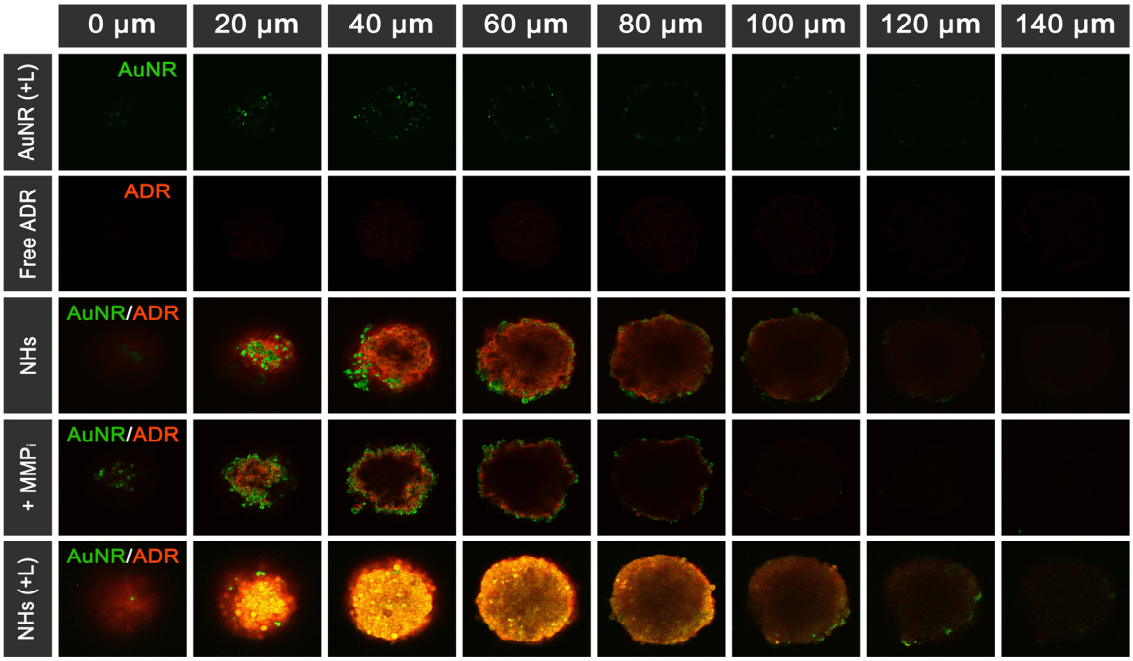


Fig. S31 2PLSM images of SKOV3/ADR MTSs incubated with AuNR (+L, 4 h post-incubation), free ADR, AuNR@DA NHs, AuNR@DA NHs (MMP inhibitor pretreated) and AuNR@DA NHs (+L, 4 h post-incubation) (AuNR: 50.0 μg mL^{-1}, ADR: 2.0 μg mL^{-1}) for 6 h.

2.23 Live and dead stain of SKOV3/ADR MTSs

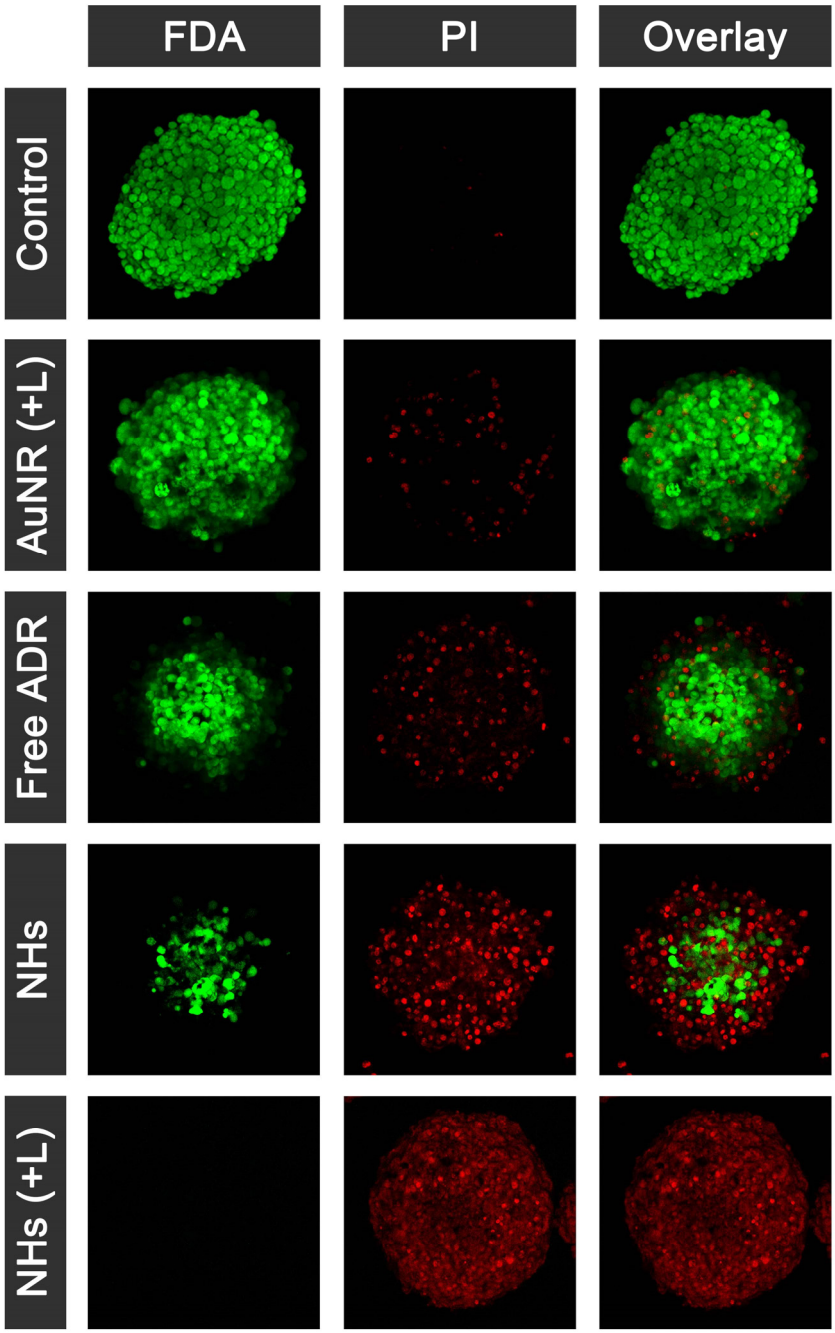


Fig. S32 Fluorescence images of SKOV3/ADR MTSs treated with AuNR (+L, 12 h post-incubation), free ADR, AuNR@DA NHs and AuNR@DA NHs (+L, 12 h post-incubation) (AuNR: 50.0 μg mL^{-1}, ADR: 2.0 μg mL^{-1}) for 24 h.

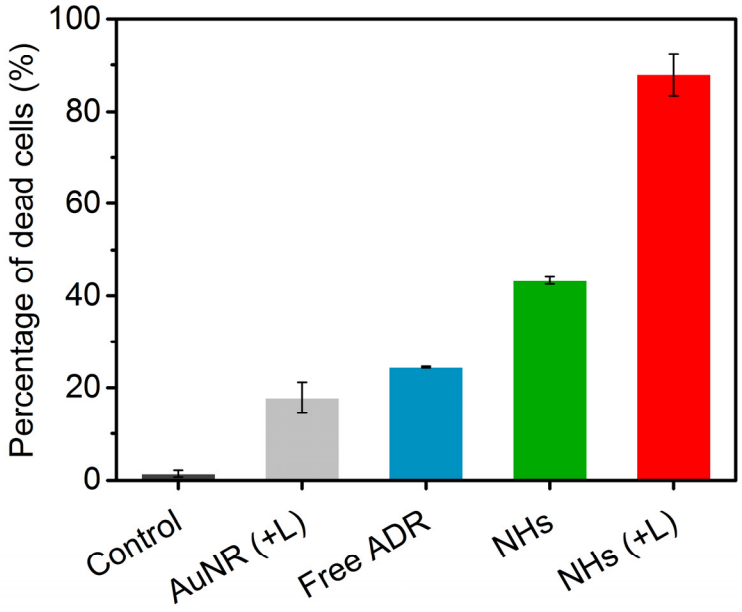


Fig. S33 Percentage of dead cells in treated SKOV3/ADR MTSs.

2.24 SKOV3/ADR MTSs growth inhibition

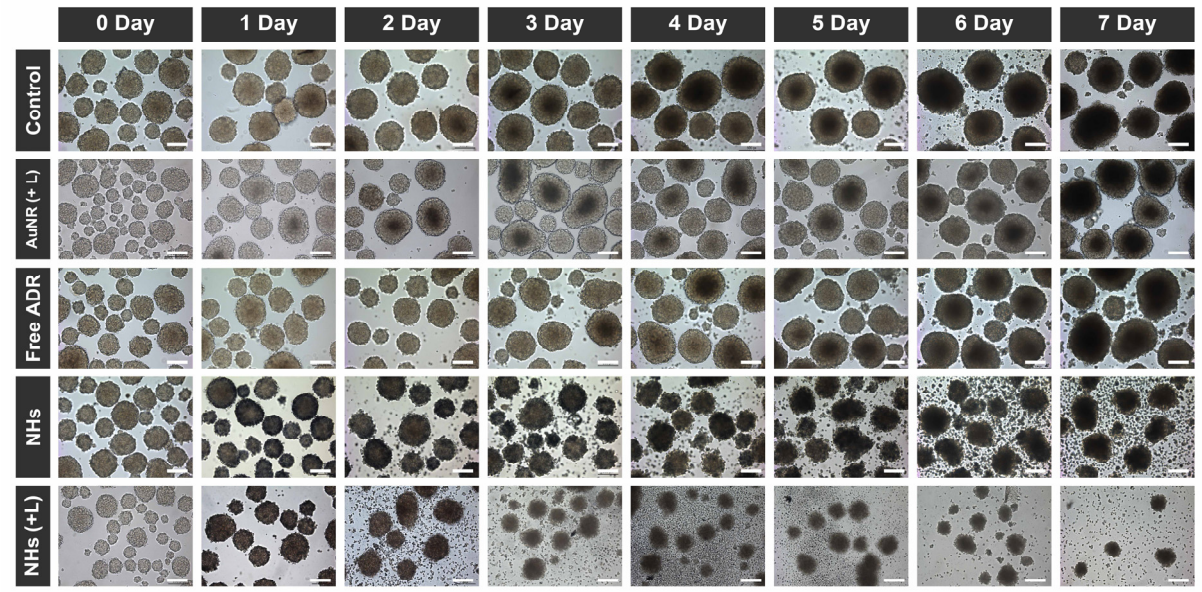


Fig. S34 Photographs of SKOV3/ADR MTs treatment with AuNR (+L, 12 h post-incubation), free ADR, AuNR@DA NHs and AuNR@DA NHs (+L, 12 h post-incubation) (AuNR: 50.0 μg mL^{-1}, ADR: 2.0 μg mL^{-1}) for 24 h. The MTSs were photographed every day for 7 days. (Scale bar: 200 μm)

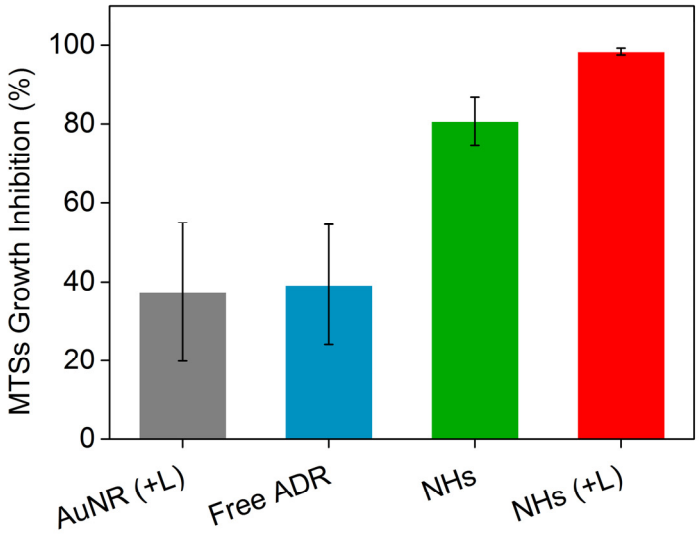


Fig. S35 MTSs inhibition ratio of SKOV3/ADR MTs treatment with AuNR (+L), free ADR, AuNR@DA NHs and AuNR@DA NHs (+L) (AuNR: 50.0 μg mL^{-1}, ADR: 2.0 μg mL^{-1}) over 7 days. (mean ± SD, n = 15)

2.25 *In vivo* photothermal imaging

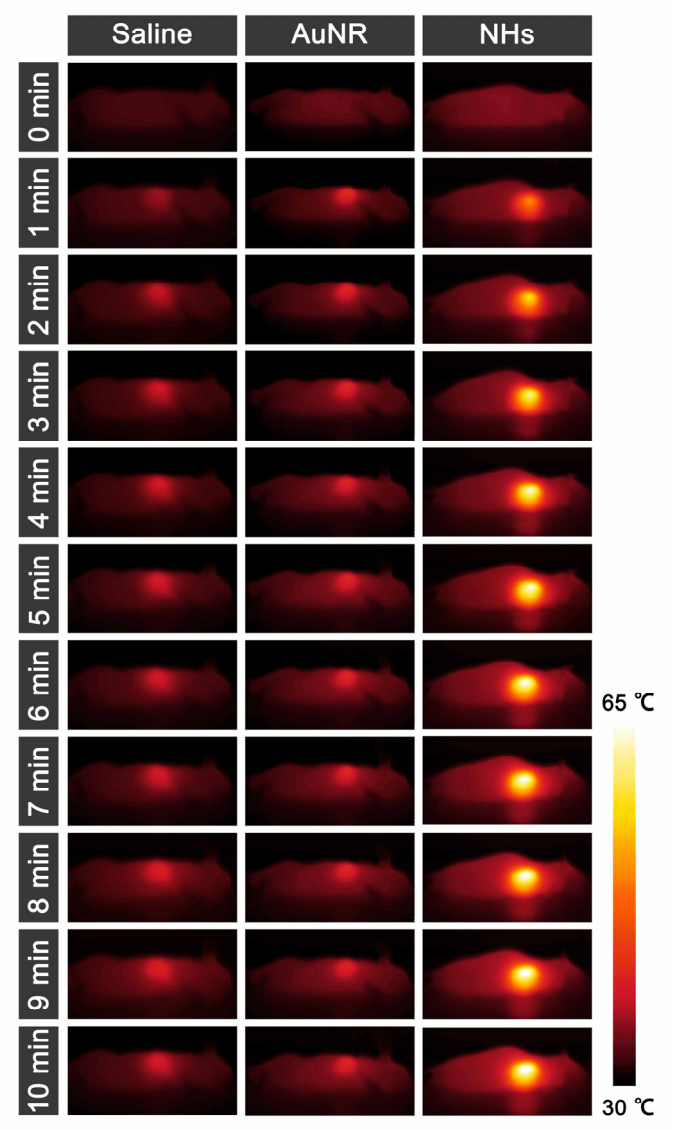


Fig. S36 *In vivo* photothermal images of tumor-bearing mice treated with saline, AuNR and AuNR@DA NHs. Laser irradiation was conducted at 6 h post-injection.

2.26 Blood perfusion assay

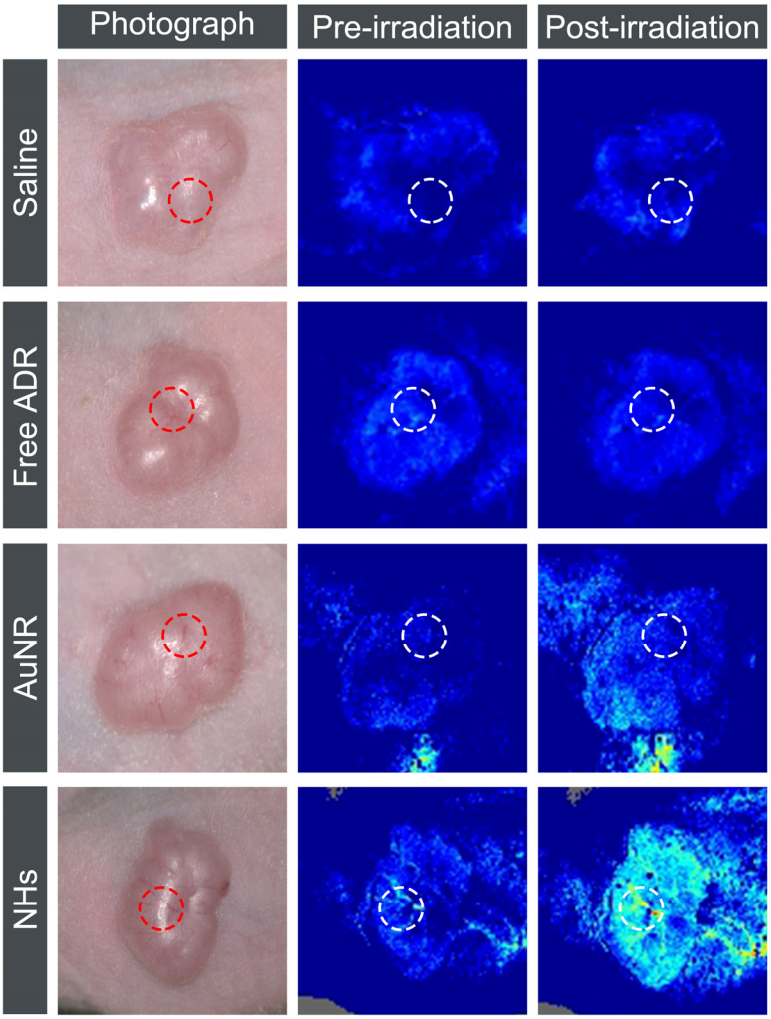


Fig. S37 LDBF images of tumor sites of mice before and post laser irradiation. NIR laser irradiation was conducted at 6 h post injection of saline, free ADR, AuNR and AuNR@DA NHs. The red and white ROI indicates the tumor site analyzed using moorFLPI software.

2.27 *In vivo* PAT imaging

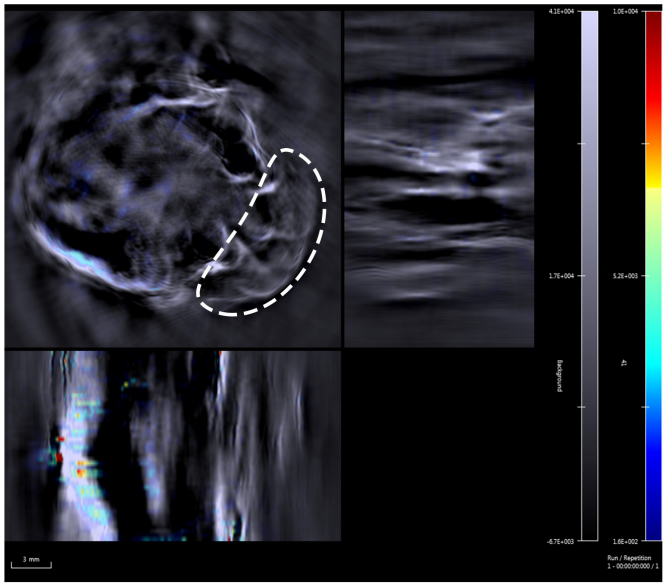


Fig. S38 PAT image of SKOV3/ADR tumor-bearing mouse without injection.

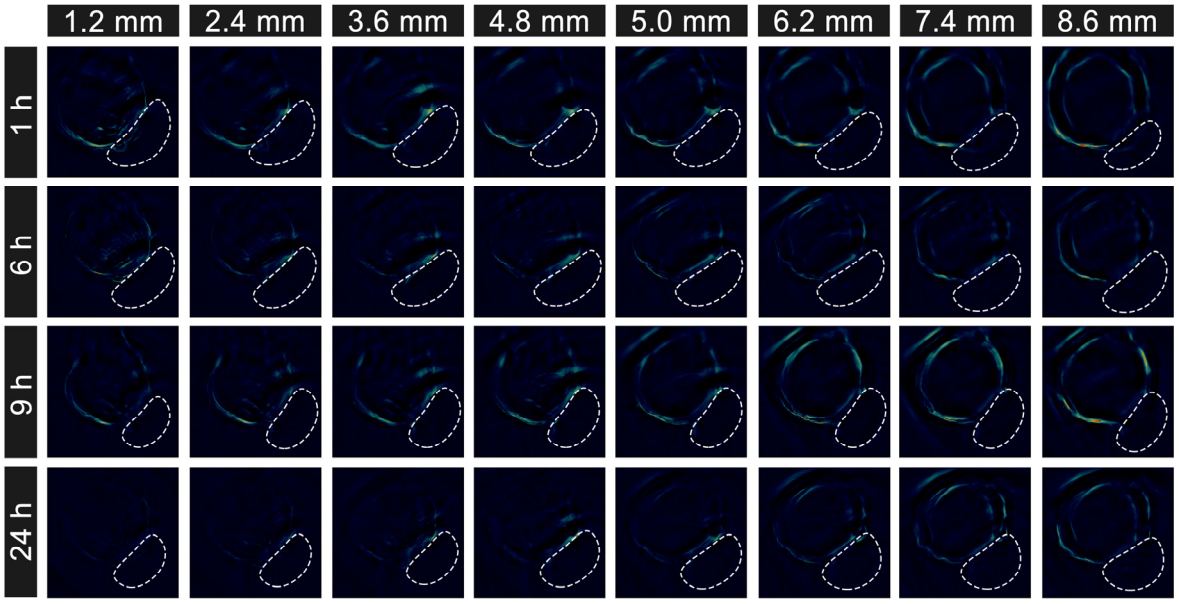


Fig. S39 *In vivo* photoacoustic imaging of SKOV3/ADR tumor-bear mouse treated with AuNR (+L). Laser irradiation was conducted at 6 h post injection.

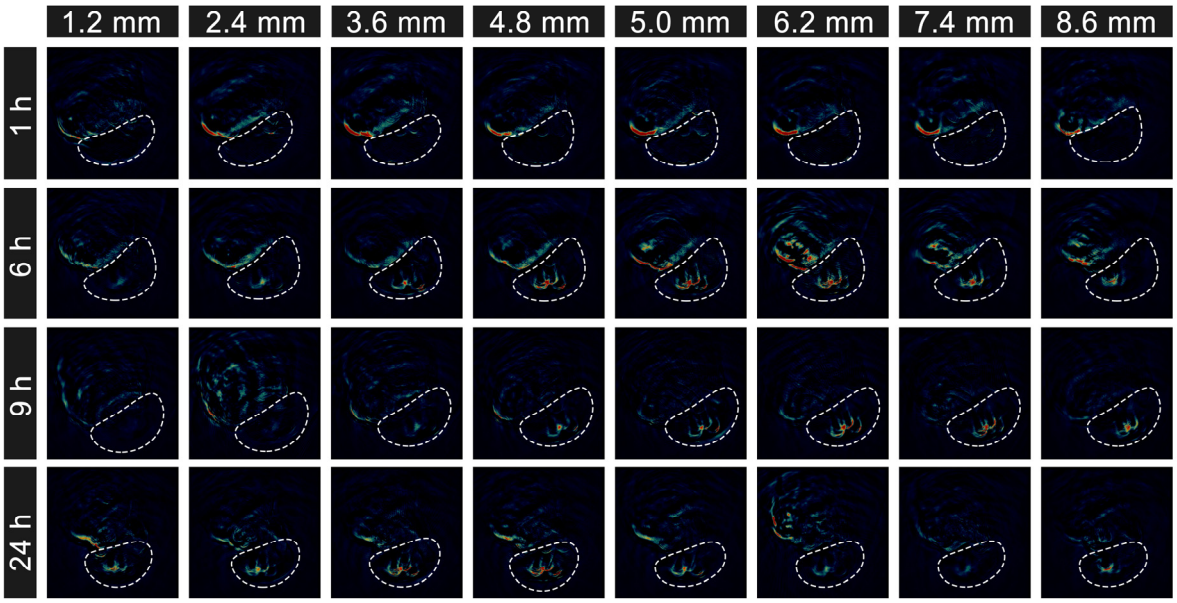


Fig. S40 *In vivo* section galleries of photoacoustic images of SKOV3/ADR tumor-bearing mouse treated with AuNR@DA NHs.

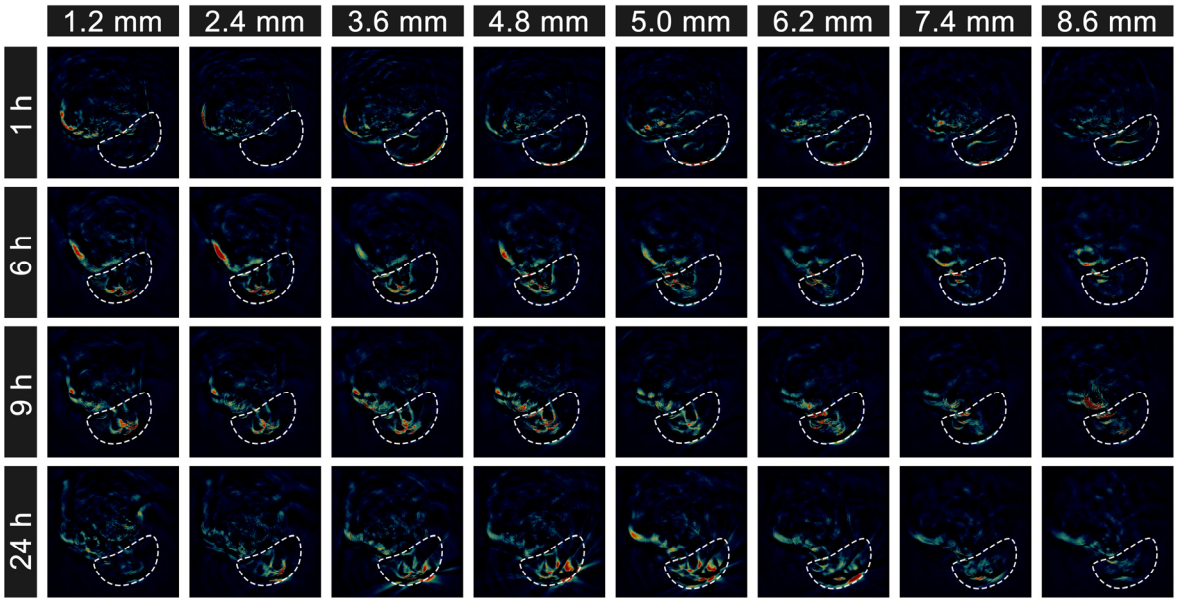


Fig. S41 *In vivo* section galleries of photoacoustic images of SKOV3/ADR tumor-bearing mouse treated with AuNR@DA NHs (+L). Laser irradiation was treated at 6 h post injection.

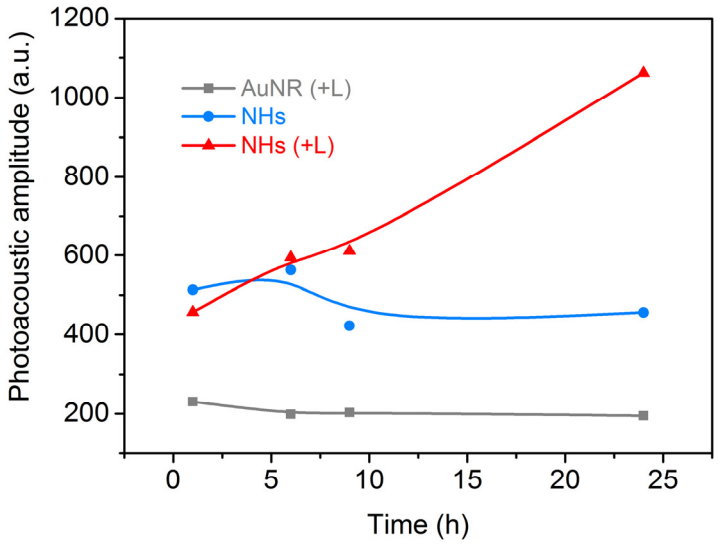


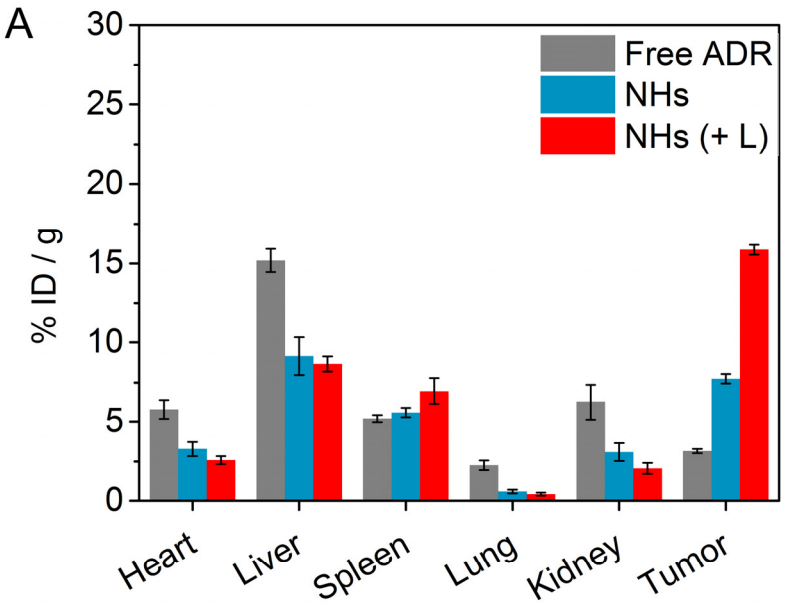
Fig. S42 Time versus mean photoacoustic intensity of tumor tissue.

2.28 Tumor accumulation of AuNR

	AuNR (% ID)	AuNR (% ID g^{-1})
AuNR (+L)	0.12 ± 0.01	1.14 ± 0.03
AuNR@DA NHs	0.78 ± 0.12	7.33 ± 0.08
AuNR@DA NHs (+L)	1.4 ± 0.07	13.76 ± 1.50

Fig. S43 Tumor accumulation of AuNR in SKOV3/ADR tumors. Tumor tissues were collected at 24 h post-injection. (mean ± SD, n = 3)

2.29 *In vivo* distribution of ADR



B

	ADR (% ID)	ADR (% ID g^{-1})
ADR	0.43 ± 0.03	3.16 ± 0.14
AuNR@DA NHs	0.83 ± 0.11	7.73 ± 0.29
AuNR@DA NHs (+L)	1.63 ± 0.10	15.86 ± 0.31

Fig. S44 (A) *In vivo* ADR distribution of free ADR, AuNR@DA NHs and AuNR@DA NHs (+L, 6 h post-injectipn). (B) ADR distribution in tumors of free ADR, AuNR@DA NHs and AuNR@DA NHs (+L, 6 h post-injectipn). The tissues were collected at 24 h post injection. (mean ± SD, n = 3)

2.30 *In vivo* penetration of AuNR and free ADR

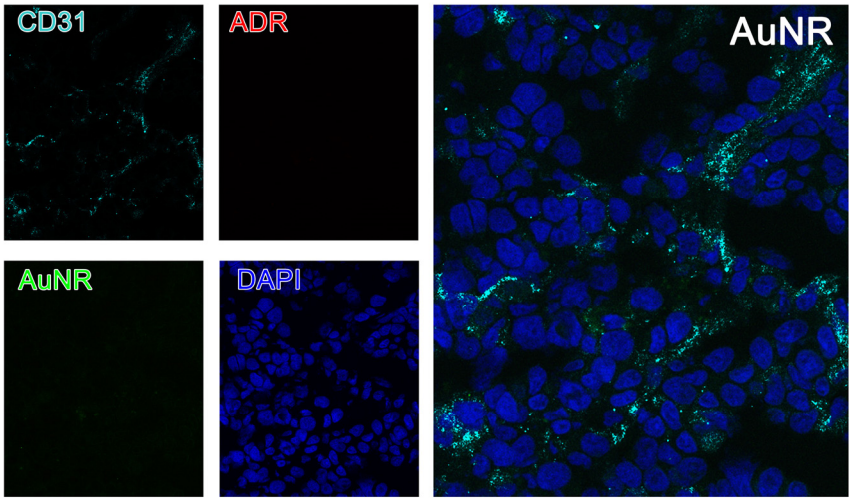


Fig. S45 2PLSM images of tumor cryo-section from AuNR (+L, 6 h post-injectipn) treatment. AuNR: 50.0 mg kg^{-1}.

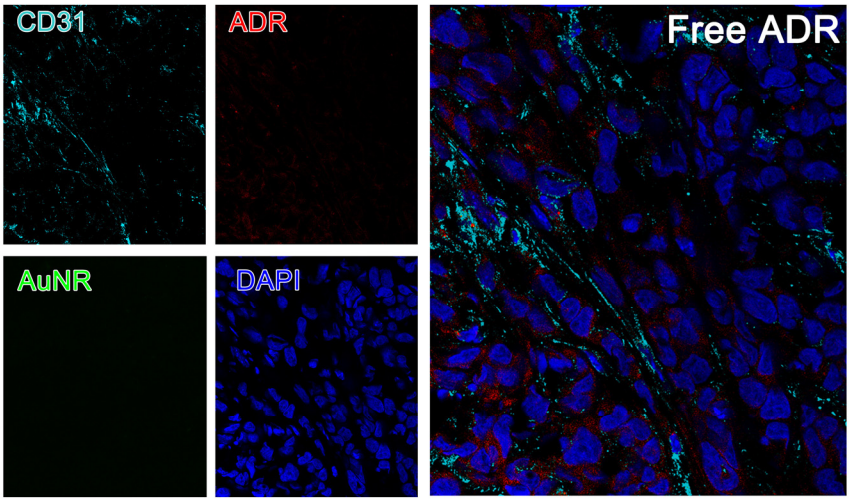


Fig. S46 2PLSM images of tumor cryo-section from ADR treatment. ADR: 2.0 mg kg^{-1}.