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Supporting Information

Peptide photowrapping of gold-silica nanocomposites to architect MMP-responsive drug capsules for chemo-photothermal therapy

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Materials. N-*α*-Fmoc-*L*-amino acids, 2-chlorotrityl chloride (CTC) resin, *o*-benzotriazole-*N*,*N*,*N*',*N*'-tetramethyluroniumhexafluorophosphate (HBTU), and *N*-hydroxybenzotriazole (HOBt) were purchased from GL Biochem. Hydrogen Tetrachloroaurate(III) Trihydrate ($HAuCl_4 \cdot 3H_2O$), tris(2,2'-bipyridiyl)dichloro ruthenium(II) hexahydrate [$Ru(bpy)_3Cl_2 \cdot 6H_2O$], ammonium persulfate, cetyltrimethylammonium bromide (CTAB), sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3$), tetraethoxysilane (TEOS), 3-aminopropyltriethoxysilane (APTES), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), N-hydroxysuccinimide (NHS), trifluoroacetic acid (TFA), triisopropylsilane (TIPS), *N*-methylmorpholine (NMM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), doxorubicin hydrochloride (DOX), 4-aminophenylmercuric acetate (APMA), and collagenase IV were purchased from Sigma-Aldrich. LIVE/DEAD viability/cytotoxicity Kit, mitotracker green, lysotracker green, and hoechst 33342 were purchased from KeyGEN Biotech. High-glucose Dulbecco's modified Eagle medium (DMEM), trypsin, fetal bovine serum (FBS), penicillin, streptomycin, phosphate-buffered saline (PBS), and trizma hydrochloric acid (Tris-HCl) were purchased from Gibco BRL. Other materials were obtained from commercial supplies and used as received.

Characterization. Transmission electron microscope (TEM) images was conducted by JEM-2100F microscope at accelerating voltage of 160 kV. High resolution mass spectrometries were performed on a ThermoScientific Q Exactive UHMR Hybrid Quadrupole-Orbitrap Mass Spectrometer. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Ascend 600 MHz spectrometer. Elemental analyses were conducted on a Carlo Erba 1106 elemental analyzer. FT-IR spectra were recorded on a Thermo Scientific Nicolet iS5 system. UV-visible (UV-vis) absorption spectra were recorded on a Shimadzu UV-2600 spectrophotometer. Fluorescence measurements were run on a Hitachi F-2500 fluorescence spectrophotometer. Dynamic light scattering (DLS) and zeta potential (ζ) was determined by a Malvern Nano-ZS90 Zetasizer.

Synthesis of MMP Cleavable Undecapeptide. The MMP cleavable undecapeptide (YYDPLGLADYY) was synthesized on CTC resin through SPPS. For each coupling of amino acid, 4 eq. of HBTU/HOBt was used. The removal of Fmoc was performed with 20% piperidine/DMF (*v*/*v*). After completion of the SPPS, the peptide was cleaved and deprotected with TFA/TIPS/H_2O (95/2.5/2.5, *v*/*v*/*v*). The crude product was purified by HPLC and lyophilized. The purity of the peptides was confirmed by analytical HPLC. HR-ESI MS: *m/z* = Calcd. 1350.5894, found 1350.5907 for $[M - H]^-$, $C_{66}H_{84}N_{11}O_{20}^-$; Calcd. 1372.5714, found 1372.5725 for $[M - 2H + Na]^-$, $C_{66}H_{83}N_{11}O_{20}Na^-$; Calcd. 1394.5533, found 1394.5546 for $[M - 3H + 2Na]^-$, $C_{66}H_{82}N_{11}O_{20}Na_2^-$. Anal. Calcd. for $C_{66}H_{85}N_{11}O_{20}$: C, 58.61; H, 6.34; N, 11.39. Found: C, 59.05; H, 6.57; N, 11.06. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ: 12.45 (br, 3H), 9.22 (br, 4H), 8.68 (m, 2H),

8.12 (d, J = 7.5 Hz, 1H), 7.95 (m, 7H), 7.59 (m, 1H), 7.00 (m, 8H), 6.65 (m, 8H), 4.83 (m, 1H), 4.31 (m, 9H), 3.70 (m, 4H), 2.86 (m, 12H), 1.86 (m, 4H), 1.58 (m, 2H), 1.44 (m, 4H), 1.37 (s, 3H), 0.86 (d, 12H).

Preparation of YY Modified Mesoporous Silica-Coated Gold Nanorods (Au@SiO_2). YY modified Au@SiO_2 was prepared according to our previous report, where amino-modified mesoporous silica-coated gold nanorods was employed as the starting material.[1] Utilizing an *in situ* grafting-cleavage strategy, protected peptide (Ac-pYY-COOH) was covalently grafted onto silica surface via EDC/NHS chemistry (YY/EDC/NHS = 1/1/2), and then deprotected by the treatment of TFA/TIPS/H_2O (95/2.5/2.5, *v*/*v*/*v*). After centrifugation and washed with deionized water, YY modified Au@SiO_2 was obtained as an aqueous redispersion for further use. Ac-pYY-COOH was prepared similarly to MMP cleavable undecapeptide through SPPS. HR-ESI MS: *m/z* = Calcd. 499.2808, found 499.2808 for $[M + H]^+$, $C_{28}H_{39}N_2O_6^+$; Calcd. 521.2628, found 521.2627 for $[M + Na]^+$, $C_{28}H_{38}N_2O_6Na^+$; Calcd. 543.2447, found 543.2441 for $[M – H + 2Na]^+$, $C_{28}H_{37}N_2O_6Na_2^+$. Anal. Calcd. for $C_{28}H_{38}N_2O_6$: C, 67.45; H, 7.68; N, 5.62. Found: C, 67.88; H, 7.44; N, 5.39. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ: 12.69 (br, 1H), 8.21 (d, J = 7.7 Hz, 1H), 7.99 (d, J = 8.6 Hz, 1H), 7.12 (dd, J = 8.3 and 4.7 Hz, 4H), 6.85 (dd, J = 14.3 and 8.4 Hz, 4H), 4.48 (m, 1H), 4.39 (m, 1H), 3.01 (m, 1H), 2.87 (m, 2H), 2.61 (m, 1H), 1.71 (s, 3H), 1.25 (s, 18H).

Photoencapsulation of DOX into Au@SiO_2@Pep. The preloading of DOX (1 mg mL^{-1}) was performed by mixing with Au@SiO_2 (0.5 mg mL^{-1}) at room temperature and then stirring in an aqueous medium for 12 h to obtain DOX loaded Au@SiO_2 (Au@SiO_2/DOX). After addition of the MMP cleavable undecapeptide YYDPLGLADYY and ruthenium photosensitizer, the system was adjusted to pH 6.5 and immediately mounted on a homemade photoreactor under visible light irradiation with 405-nm light-emitting diodes (LEDs) (112 mW cm^{-2}, 50 mA direct current (DC) input) for 10 min.[2] As completion of photoencapsulation, the Au@SiO_2/DOX@Pep nanocapsules were washed thoroughly and collected by centrifugation. The DOX loading amount in nanocapsules was calculated by subtracting the free DOX in the supernatant using a calibration plot at 480 nm. The blank Au@SiO_2@Pep nanocapsules were prepared parallelly for comparison.

***In Vitro* Drug Release of Au@SiO_2/DOX@Pep.** For enzyme-responsive drug release study, Au@SiO_2/DOX@Pep nanocapsules (1 mg, with equivalent dose of 108 μg DOX) were incubated in 1 mL Tris-HCl buffer (pH 6) at 37°C in the presence of collagenase IV (MMP-2/9), respectively. At designated time points, the samples were exposed to an 808-nm NIR laser (Beijing Viasho Technology Co., China) irradiation (1.0 W cm^{-2}, 10 min) and the supernatant was extracted to quantify the amount of released DOX by UV-vis spectrometer. Collagenase IV (0.5 mg mL^{-1} in 50 mM Tris-HCl buffer) was activated with the 2.5 mM APMA solution at 37°C for 1 h prior to experiment. For comparison with either

enzyme-reposive or photothermal-triggered system, 1 mL of samples was treated with merely enzymatic incubation or NIR laser irradiation to test the DOX release.

Measurement of Photothermal Effect. Different concentrations of Au@SiO_2@Pep nanocapsules dispersions in PBS were prepared in the centrifuge tubes and exposed to an 808-nm NIR laser at a power density of 1.0 W cm^{-2} for 15 min. The temperature fluctuations of samples were recorded on an infrared thermal camera (Fotric 225) with 1-min time intervals, along with the measurement of UV-vis absorption of Au@SiO_2@Pep. The photothermal stability of Au@SiO_2@Pep was measured by cycle irradiation, the sample (180 μg mL^{-1}) was swithched between exposure to NIR and followed cooling to room temperature for 4 times.

Cytocompatibility. Human alveolar basal epithelial cells (A549), human hepatocellular carcinoma cells (HepG2), and human umbilical vein endothelial cells (HUVEC) were selected to study the biocompatibility of the nanocapsules. All cells were obtained from KeyGEN Biotech and cultured in DMEM containing 10% FBS and 1% penicillin-streptomycin. Growth conditions were maintained as 37°C, 5% CO_2 and 95% humidity. Cytocompatibility was assessed using the MTT assay. Briefly, cells growing in log phase were seeded into a 96-well cell-culture plate at a density of 5×10^3/well. As incubated overnight, Au@SiO_2@Pep was added with various concentrations of 1.25, 2.5, 5, 10, 20, 40, 80, and 160 μg ml^{-1}. After parallel incubation for 24 h and 48 h, the cells were treated with 25 μL of MTT solution (5 mg mL^{-1} in PBS) and incubated for additional 4 h. Following incubation, 100 μL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals, and absorbance was recorded at 490 nm on an Enspire multimode microplate reader (PerkinElmer). The cell viability can be calculated as the ratio of the mean absorbance of treatment group *vs*. control group. MTT assays of cells incubated in the medium without nanocapsules were set as control.

***In Vitro* Anticancer Study.** *In vitro* anticancer activity was assessed by MTT assay using A549 and HepG2 cells. Cells were seeded into 96-well plates at a density of 5×10^3/well and tested against Au@SiO_2/DOX@Pep at various concentrations (1.25, 2.5, 5, 10, 20, 40, 80, and 160 μg mL^{-1}) with equivalent DOX (0.14, 0.27, 0.54, 1.08, 2.16, 4.32, 8.64, and 17.28 μg mL^{-1}). Collagenase IV was added to the system to cleave the peptide shell and activate the nanocapsules. After 8 h incubation, the cells were exposed to a NIR laser (1.0 W cm^{-2}) for 10 min, and then incubated for another 24 h. Subsequently, 20 μL of MTT solution was added to each well. After 4 h, formed formazan crystals were dissolved by adding 100 μL of DMSO, and the absorbance was recorded at 490 nm using microplate reader. The parallel experiments were designed and peformed for Au@SiO_2@Pep, Au@SiO_2/DOX, Au@SiO_2/DOX@Pep, Au@SiO_2/DOX@Pep + MMP, and Au@SiO_2@Pep + NIR.

Live/dead Cell Assay. For live and dead cells observations, A549 and HepG2 cells were seeded in 35-mm glass bottom confocal dishes at a density of 1×10^5/well. After cultured overnight, the cells were treated with Au@SiO_2/DOX@Pep (80 μg mL^{-1}), and collagenase IV was added to the system to cleave the peptide shell and activate the nanocapsules. After 8 h incubation, the cells were exposed to NIR laser for 10 min, and then incubated for another 24 h. Subsequently, the cells were co-stained with calcein-AM/PI for 15 min and imaged on a confocal laser scanning microscope (CLSM, Leica TCS-SP8). The control sets were conducted for PBS + NIR and Au@SiO_2/DOX@Pep + MMP.

Cellular Uptake and Intracellular Localization. A549 cells were seeded in glass bottom dishes at a density of 1×10^5/well. After cultured overnight, the cells were treated with Au@SiO_2/DOX@Pep (40 μg mL^{-1}), and collagenase IV was added to the system to cleave the peptide shell and activate the nanocapsules. After 8 h incubation, the cells were exposed to NIR laser for 10 min, then stained with LysoTracker Green and Hoechst 33342 and imaged directly by CLSM. The control sets were conducted for DOX, Au@SiO_2/DOX@Pep, and Au@SiO_2/DOX@Pep + MMP.

Animals and Tumor Model. Female BALB/c mice at 6 weeks of ages, ~20 g, were provided by the Animal Center of Capital Medical University (CCMU). All animal experiments were performed following the protocols evaluated and approved by the Animal Ethics Committee of CCMU. The animals were housed with a 12h light/dark cycle at 22 °C and provided food and water ad libitum. To establish tumor model, CT26 cells (1×10^6) was first injected in the right flank of a mouse subcutaneously. As the tumor reached to 1~2 cm, tumor tissue was collected and cut into pieces about 1 mm and injected into the right hindlimb of mice subcutaneously. The tumors were gently pinched to measure dimensions, the size was determined by a vernier caliper and calculated as the volume (=length × $width^2/2$). As the tumor loci have been completely ablated, the volume was recorded as zero.

***In Vivo* Fluorescent Imaging.** For fluorescent imaging, NIR fluorophore, rhodamine 800 (Rh800)-loaded Au@SiO_2@Pep (Au@SiO_2/Rh800@Pep) was prepared similar as that of Au@SiO_2/DOX@Pep. Tumor-bearing mice were injected intravenously with 2 mg kg^{-1} of Au@SiO_2/Rh800@Pep. After anesthetized with isoflurane, the mice were imaged in a PerkinElmer imaging system at 1, 4, 8, 12, and 24 h post-injection (Figure S11a). For biodistribution analysis of Au@SiO_2/Rh800@Pep, the mice were sacrificed at 24 h, and the representative organs including heart, liver, spleen, lung, kidney, and tumor tissue were excised for *ex vivo* imaging (Figure S11b).

***In Vivo* Photothermal Imaging.** For photothermal imaging, tumor-bearing mice were administered with 2 mg kg^{-1} of Au@SiO_2@Pep. After 8 h post-injection, tumors were exposed to NIR laser irradiation and the real-time thermal images were recorded on an infrared thermal camera.

***In Vivo* Antitumor Activity and Biosafety.** When the tumor volumes reached approximately 50 mm^3, the mice were divided randomly into seven groups (n = 5) and treated with (I) PBS, (II) PBS + NIR, (III) DOX (2 mg kg^{-1}), (IV) Au@SiO_2/DOX (10 mg kg^{-1}), (V) Au@SiO_2/DOX@Pep (10 mg kg^{-1}), (VI) Au@SiO_2@Pep (9 mg kg^{-1}) + NIR, and (VII) Au@SiO_2/DOX@Pep (10 mg kg^{-1}) + NIR, respectively. After 8 h postinjection, tumor sites of mice in groups II, VI and VII were exposed to NIR laser for 10 minutes. The body weight and the tumor volume of each mouse were determined every other day after treatment and measured up to 14 days. At the end of the therapeutic process, tumors and major organs were excised from mice and stained with hematoxylin and eosin (H&E) for histology analysis.

Statistical Analysis. Data were presented as mean ± standard deviation and analyzed using two-tailed Student's t test. Statistical significance was noted as follows: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Table S1. Zeta potentials of bare Au@SiO_2, amino modified Au@SiO_2, YY modified Au@SiO_2, Au@SiO_2@Pep, and Au@SiO_2@Pep after MMP cleavage.

Sample	Zeta potential (mV)
bare Au@SiO_2	−21.3 ± 1.8
amino modified Au@SiO_2	9.1 ± 0.2
YY modified Au@SiO_2	−4.8 ± 2.9
Au@SiO_2@Pep	−35.4 ± 0.2
Au@SiO_2@Pep + MMP	−17.9 ± 1.1

Measured in 10 mM PBS (pH 7.4) with the concentration of 5 mg mL^{-1} at 298 K, data represent the mean ± standard deviation (n = 3).

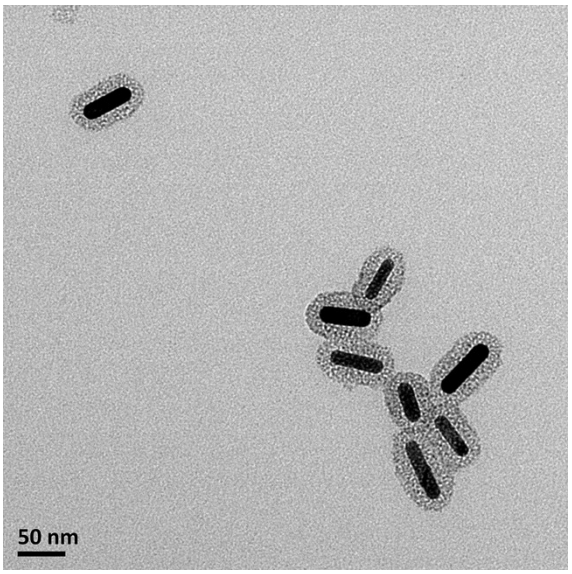

Figure S1. TEM image of Au@SiO_2.

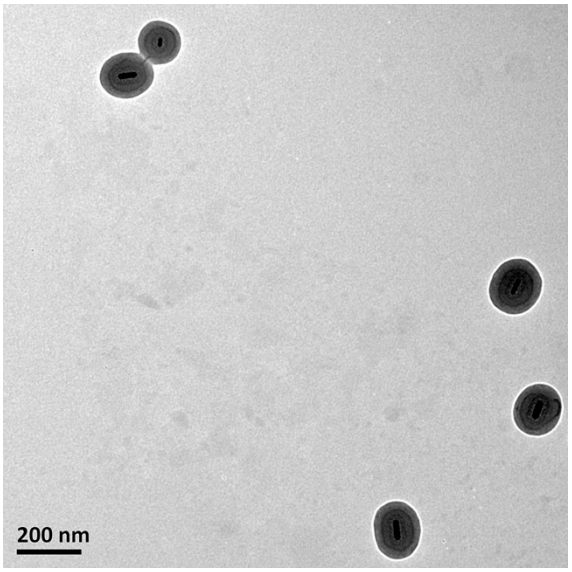

Figure S2. TEM image of Au@SiO_2/DOX@Pep.

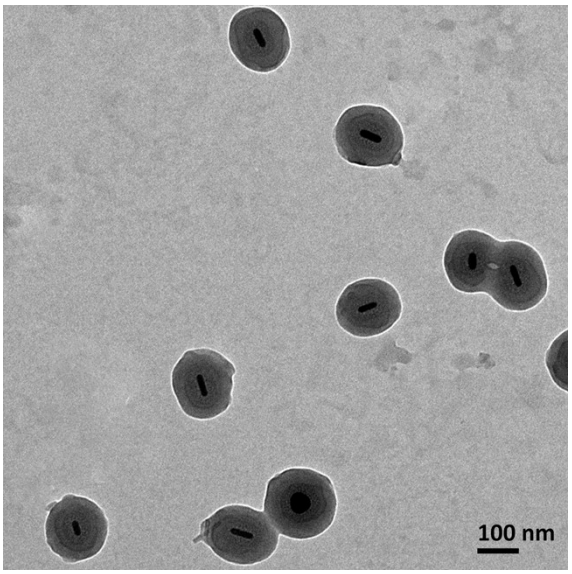

Figure S3. TEM image of Au@SiO_2/DOX@Pep after NIR irradiation.

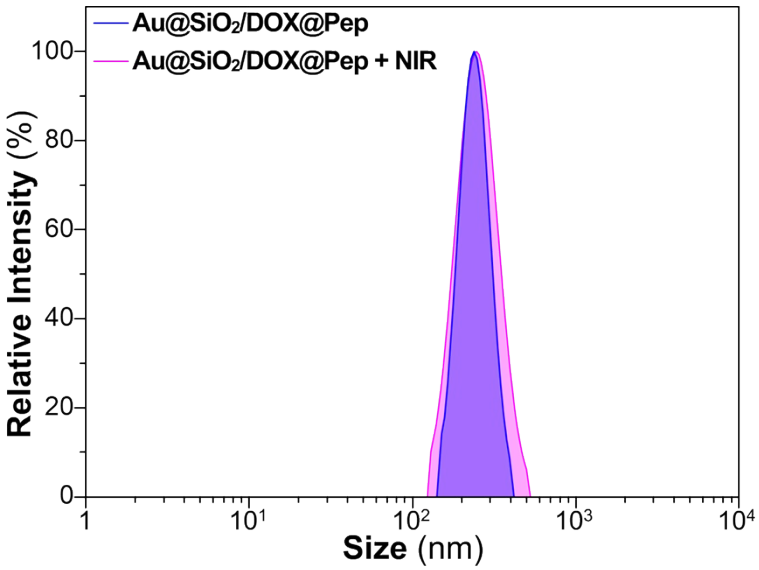

Figure S4. DLS of $Au@SiO_2$/DOX@Pep before and after NIR irradiation.

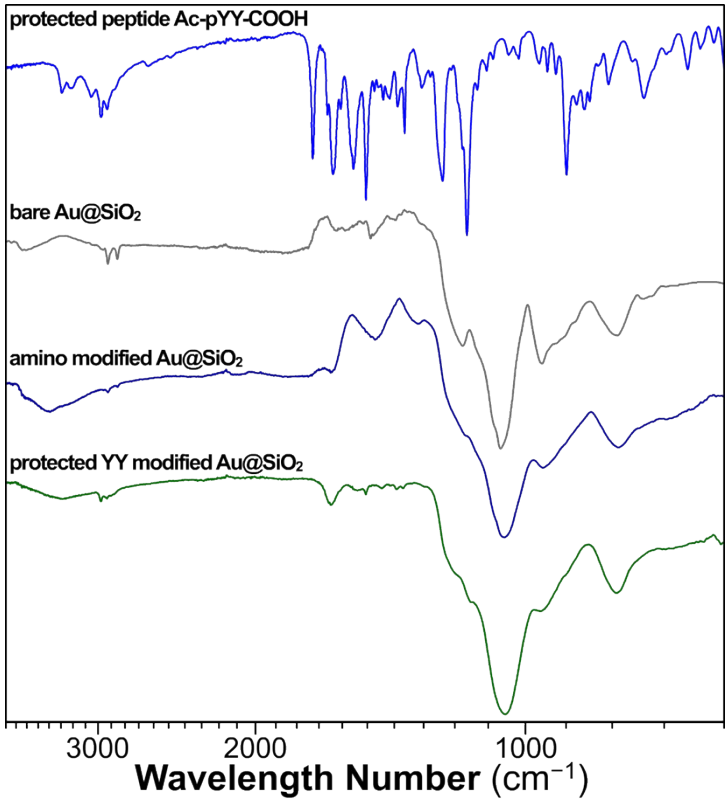

Figure S5. FT-IR spectra for protected peptide Ac-pYY-COOH, bare $Au@SiO_2$, amino modified $Au@SiO_2$, and protected YY modified $Au@SiO_2$.

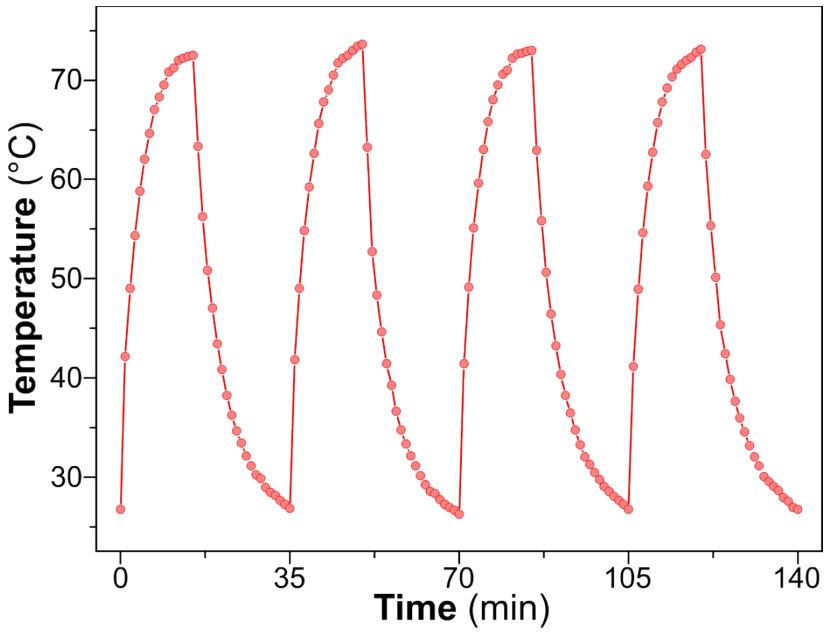

Figure S6. Temperature variation of Au@SiO_2@Pep over 4 on/off cycles of 808 nm laser excitation.

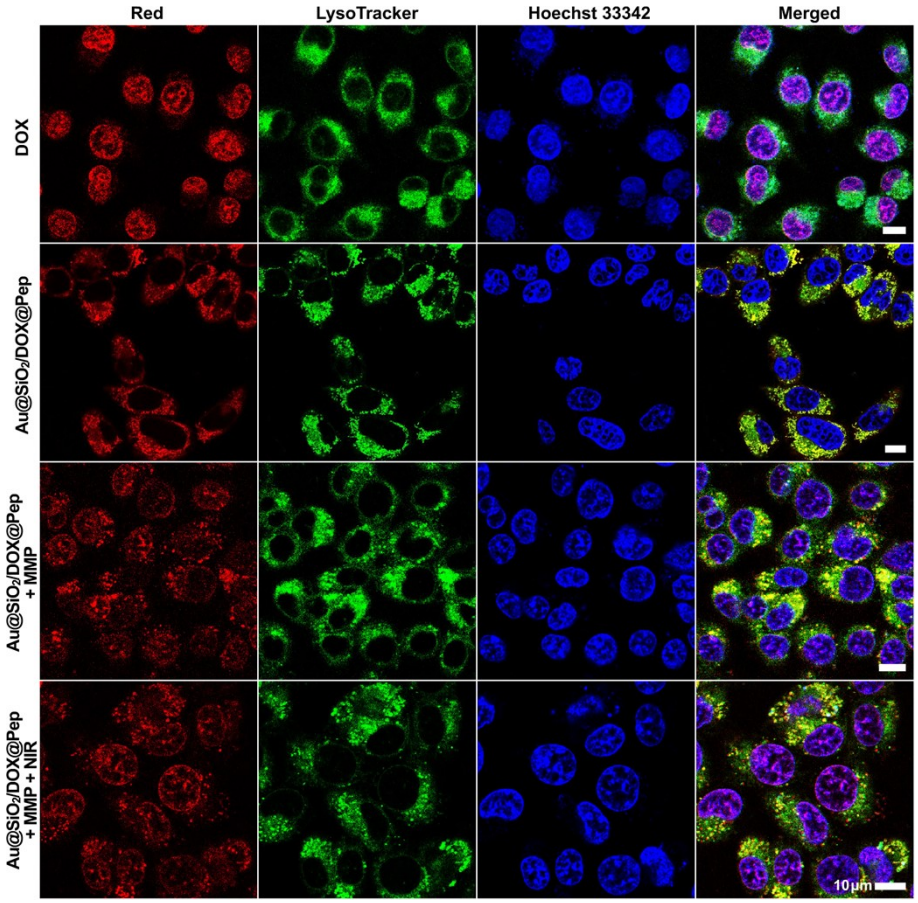

Figure S7. CLSM images for A549 cells incubated with free DOX and Au@SiO_2/DOX@Pep upon stimuli of MMP and NIR.

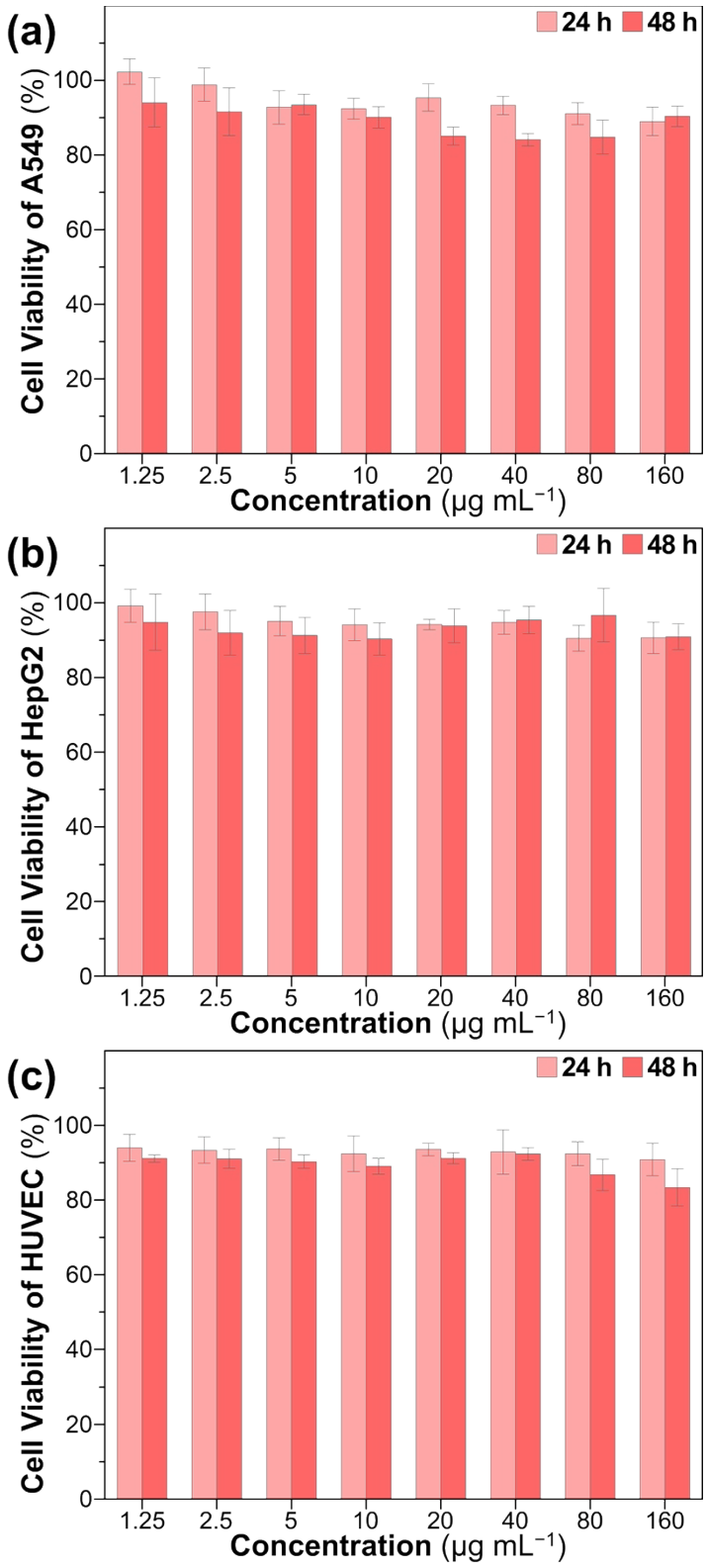

Figure S8. Cell viabilities of Au@SiO_2@Pep at various concentrations in A549 (a), HepG2 (b), and HUVEC (c) cells after 24 and 48 h incubation (n = 3).

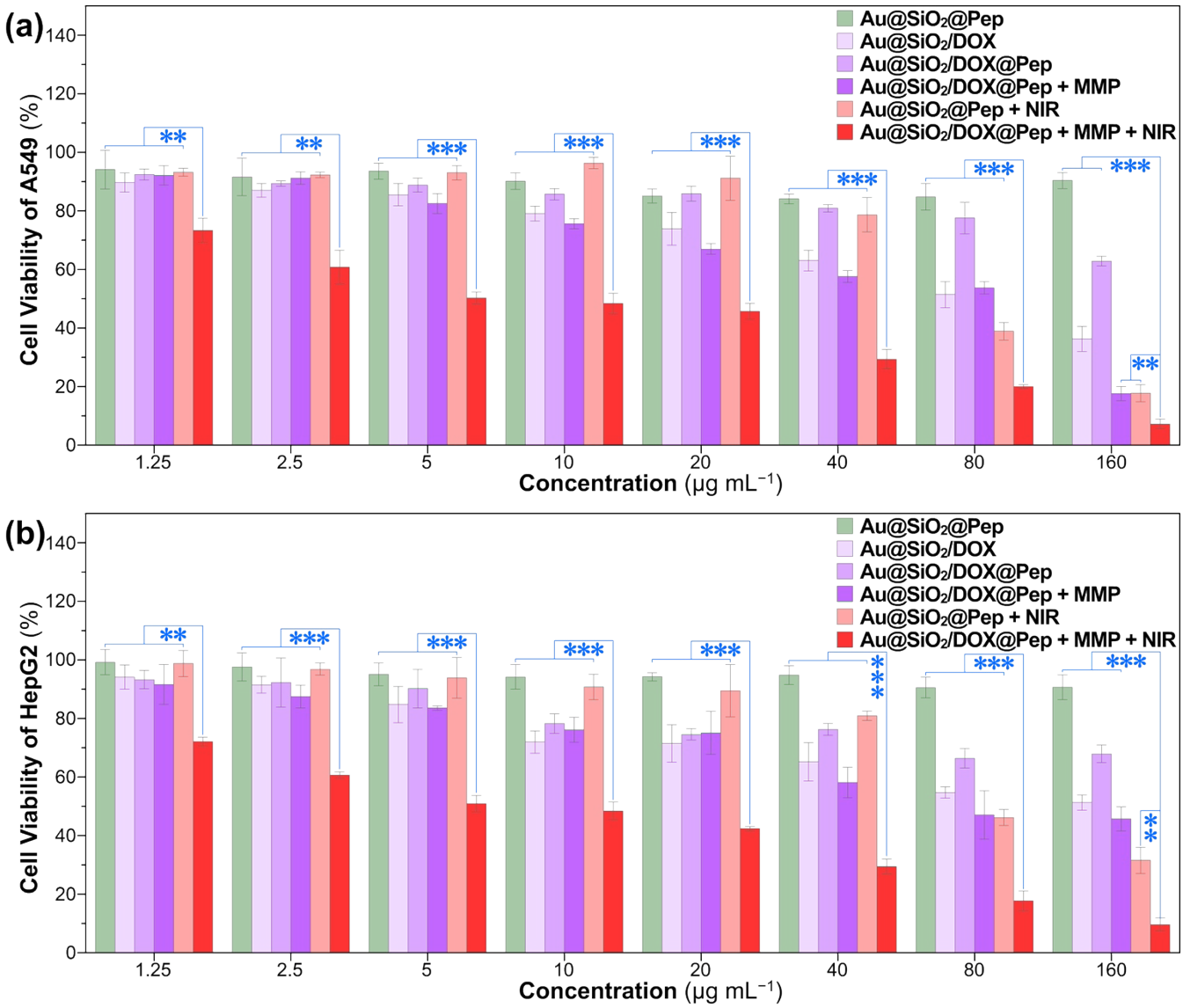

Figure S9. Viability of A549 and HepG2 cells treated with Au@SiO_2@Pep, Au@SiO_2/DOX, Au@SiO_2/DOX@Pep, Au@SiO_2/DOX@Pep + MMP, Au@SiO_2@Pep + NIR, Au@SiO_2/DOX@Pep + MMP + NIR at various concentrations (n = 3). **, $p < 0.01$; ***, $p < 0.001$.

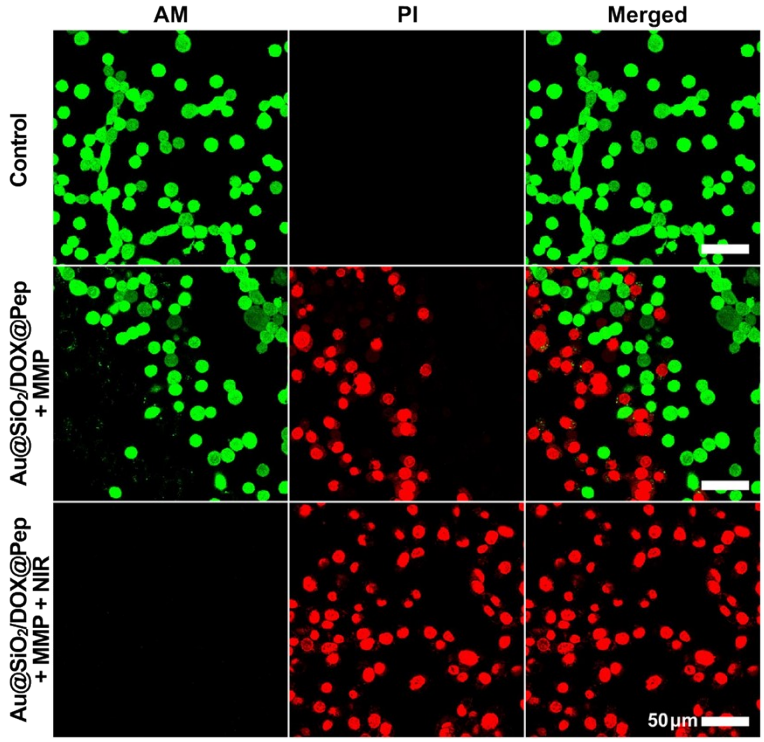

Figure S10. Live/dead staining of A549 cells incubated with PBS + NIR (control) and Au@SiO_2/DOX@Pep after stimulation of MMP or NIR/MMP.

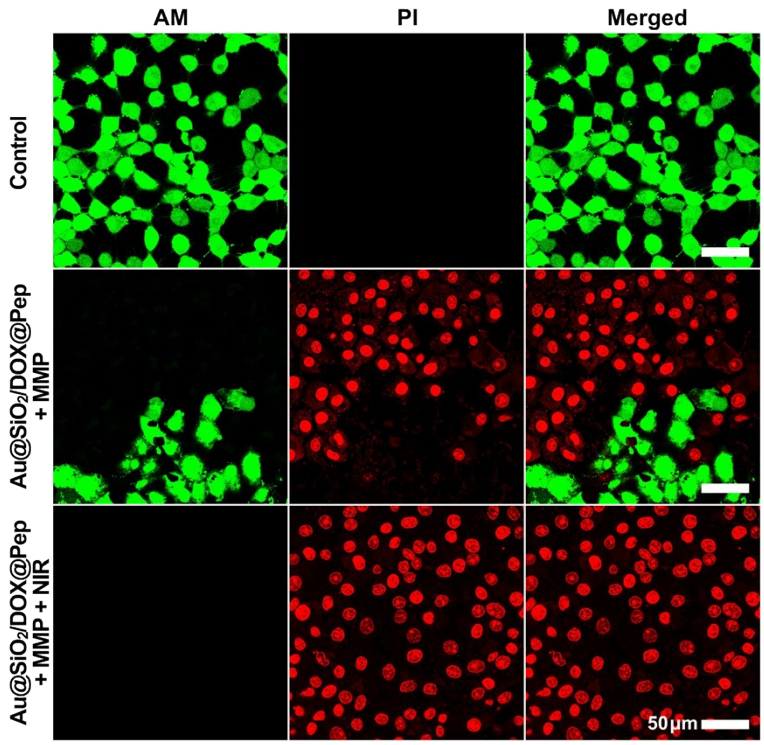

Figure S11. Live/dead staining of HepG2 cells incubated with PBS + NIR (control) and Au@SiO_2/DOX@Pep after stimulation of MMP or NIR/MMP.

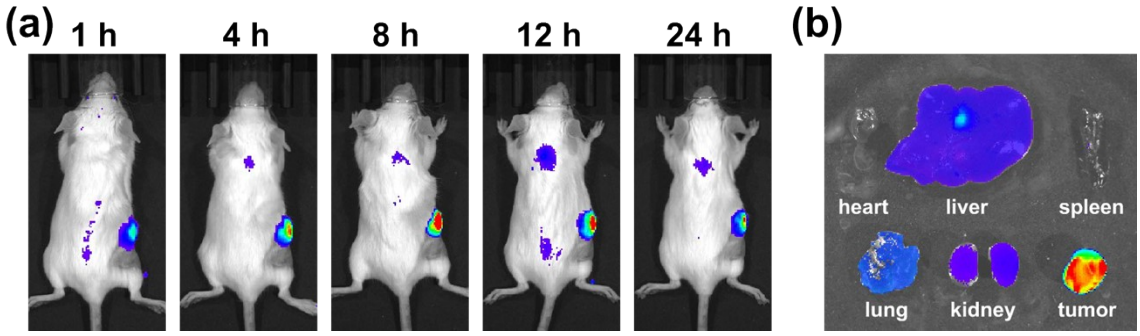

Figure S12. (a) *In vivo* fluorescence images of tumor-bearing mice treated with Au@SiO_2/Rh800@Pep at 1, 4, 8, 12, and 24 h post-injection. (b) *Ex vivo* tissue fluorescence images of the mice at 24 h post-injection.

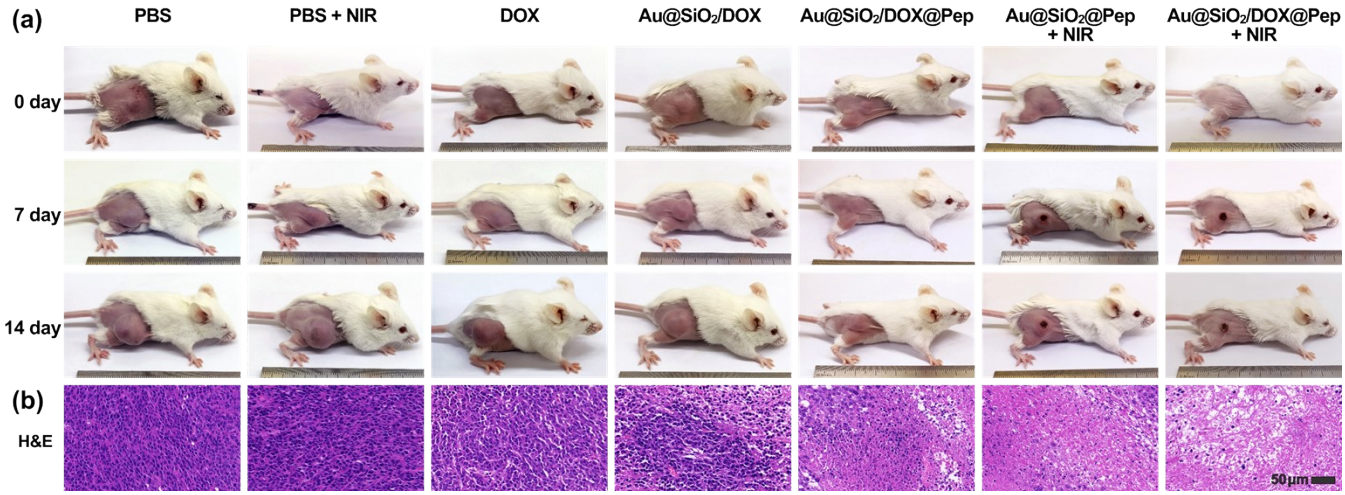

Figure S13. (a) Photographs of tumor-bearing mice treated with PBS, PBS + NIR, DOX, Au@SiO_2/DOX, Au@SiO_2/DOX@Pep, Au@SiO_2@Pep + NIR, and Au@SiO_2/DOX@Pep + NIR at different time intervals (n = 5). (b) H&E-stained tissue sections of tumors after different treatments.

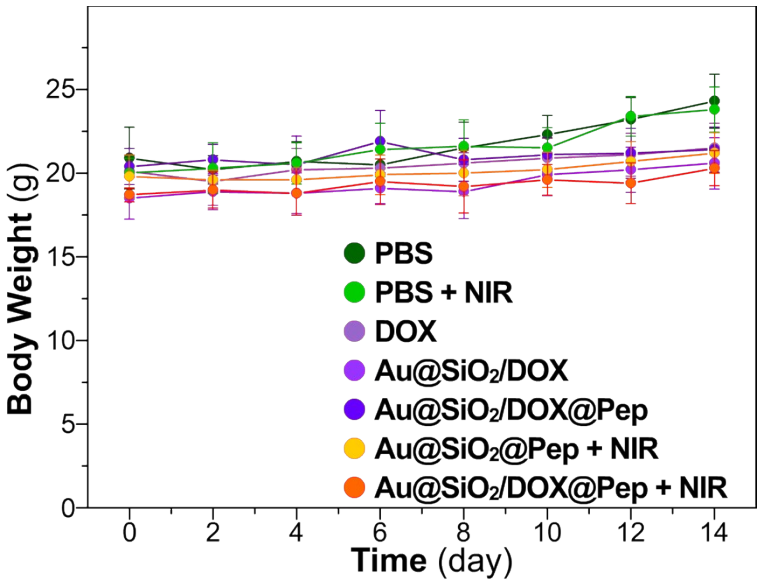

Figure S14. body weight changes of mice in different groups.

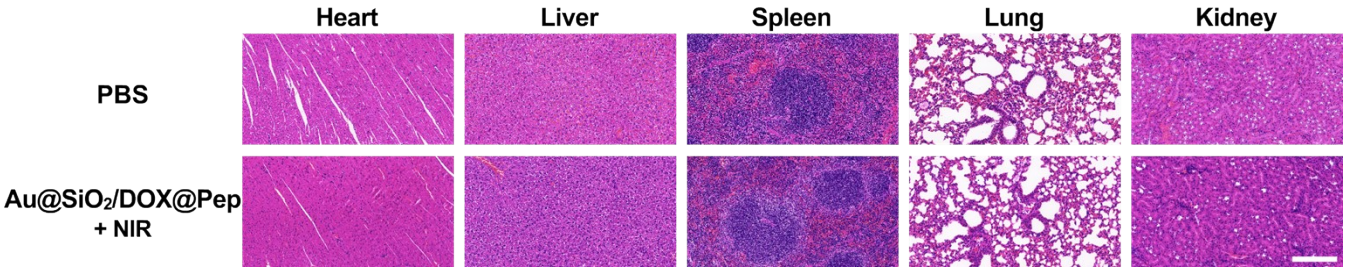

Figure S15. H&E-stained tissue sections of major organs from mice treated with PBS (control) and Au@SiO_2/DOX@Pep + NIR, scale bar presents 200 μm.

Analysis Name:		Acquisition Date:	20201109
Comment:	ESI Negative	Operator:	TIPC

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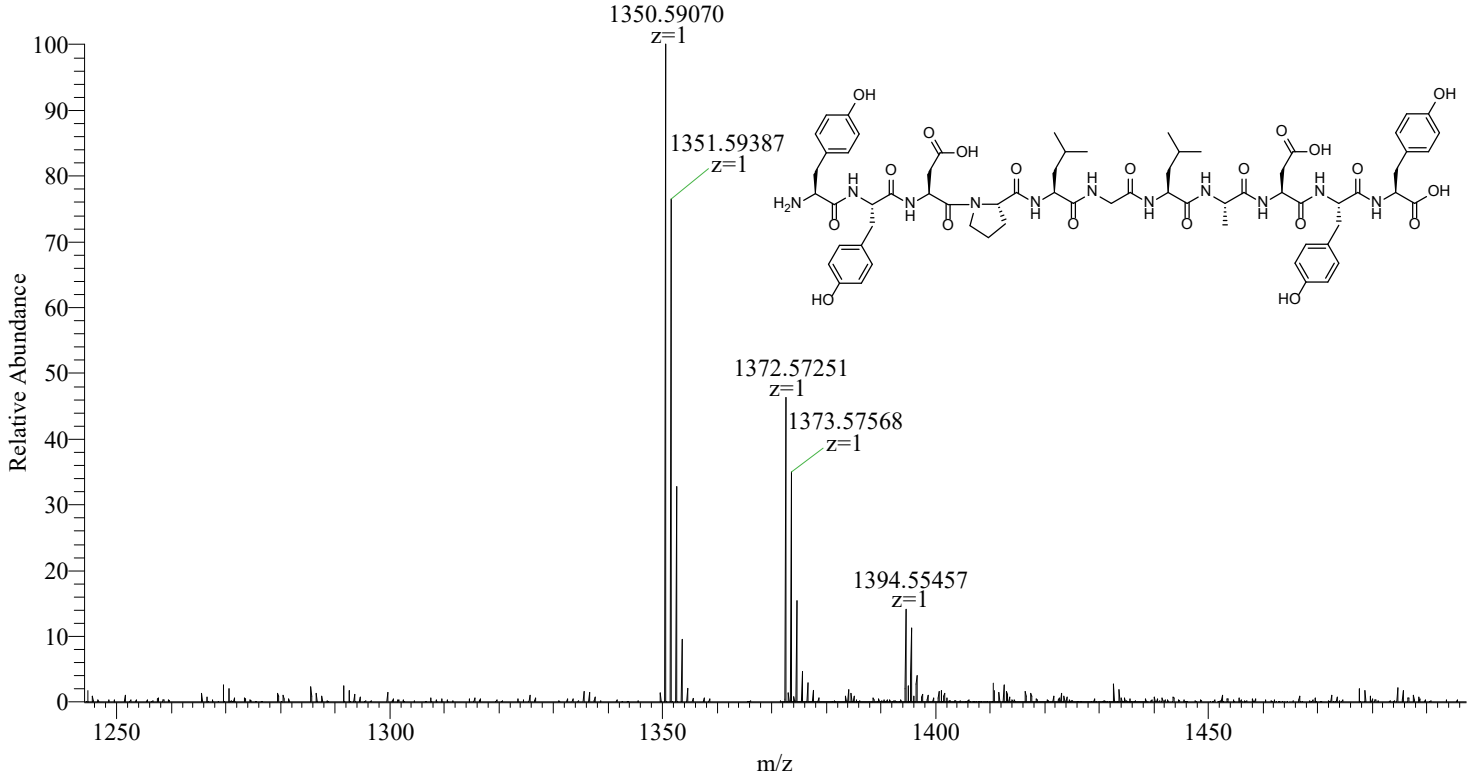

Figure S16. HR-ESI MS spectrum for MMP cleavable undecapeptide YYDPLGLADYY.

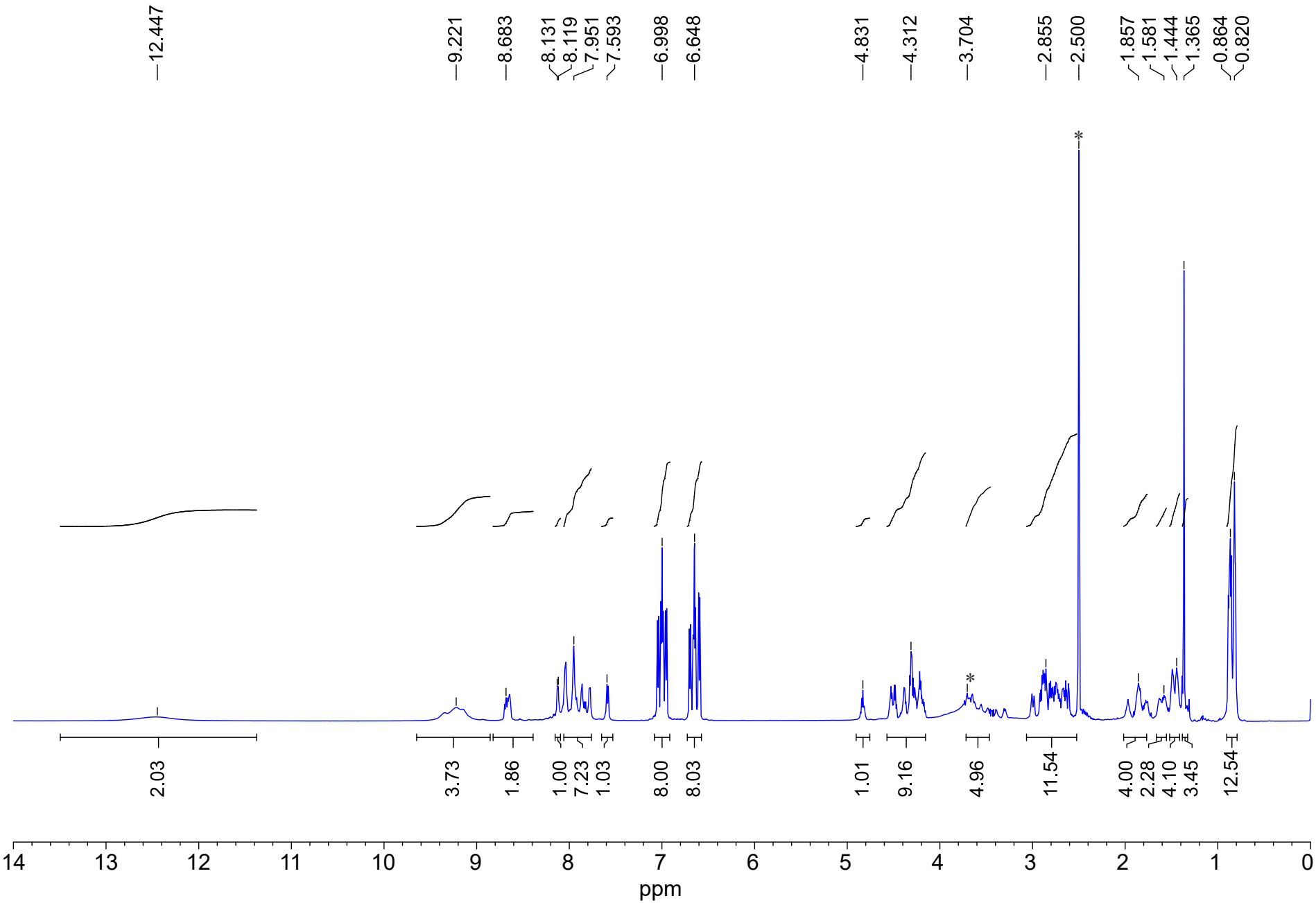

Figure S17. [1]H NMR spectrum for MMP cleavable undecapeptide YYDPLGLADYY in DMSO-d_6.

Analysis Name:		Acquisition Date:	20201109
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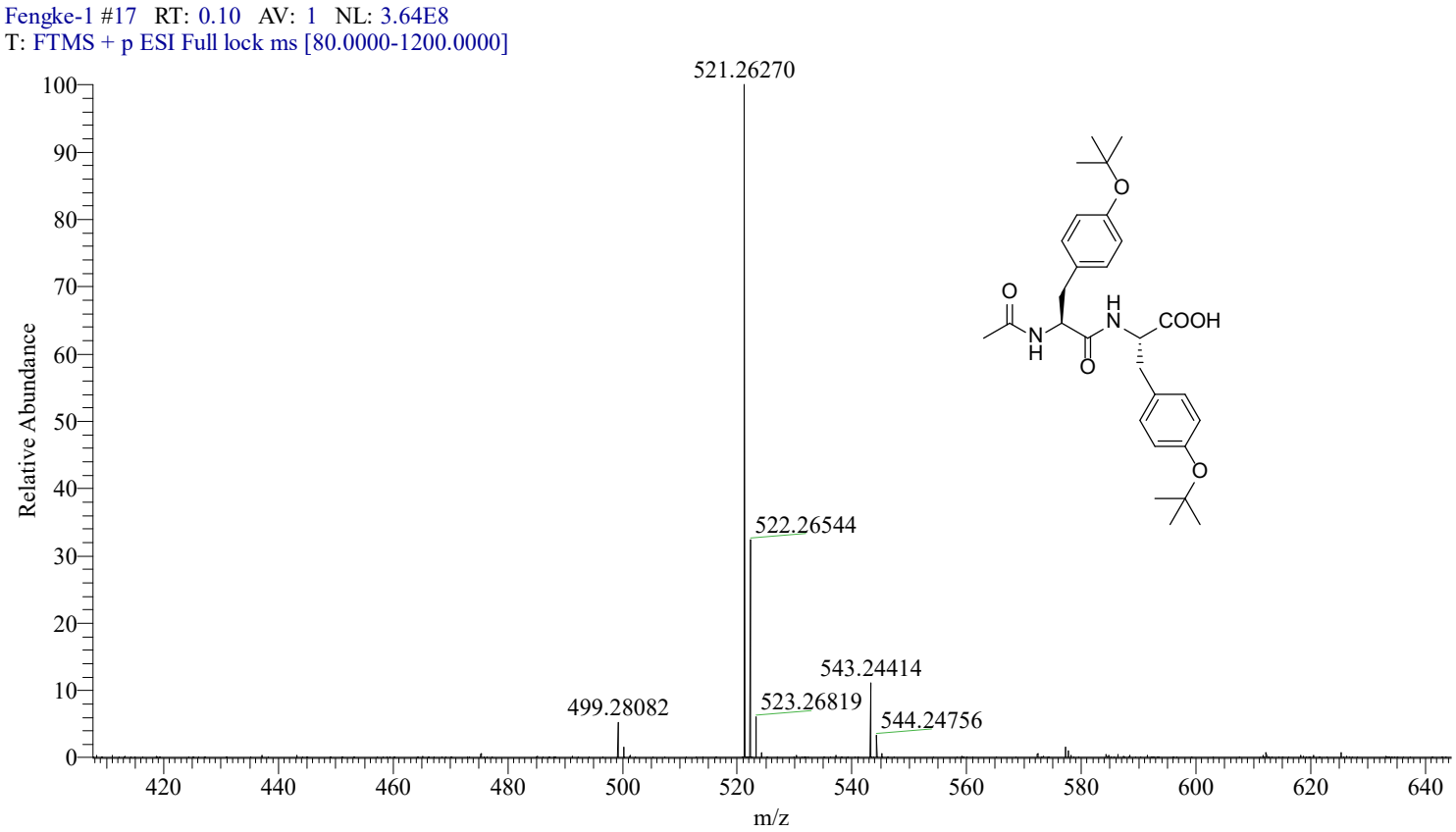

Figure S18. HR-ESI MS spectrum for protected Ac-pYY-COOH peptide.

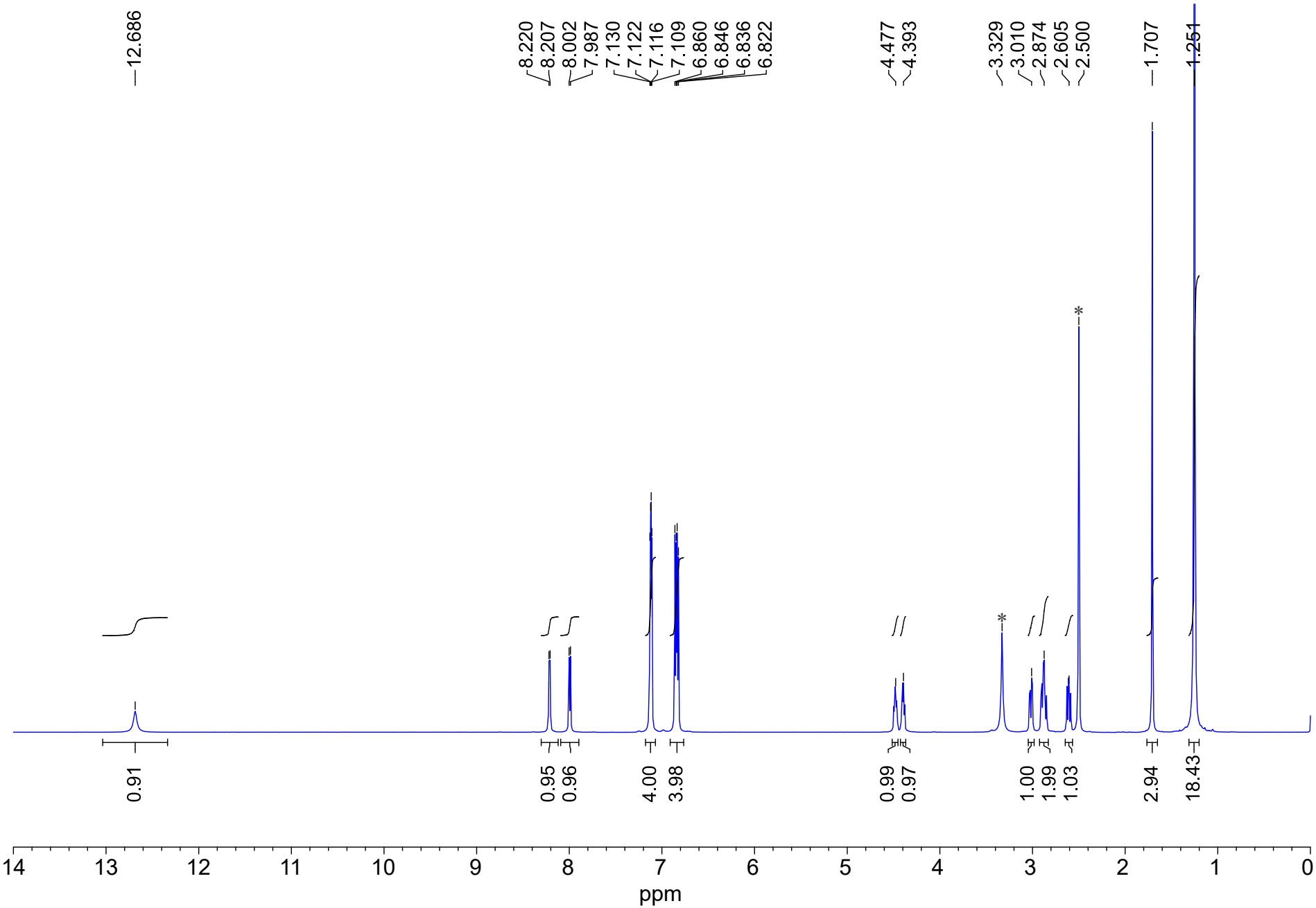

Figure S19. ^{1}H NMR spectrum for protected Ac-pYY-COOH peptide in DMSO-d_6.

References

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