Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Photocontrollable Thermosensitive Chemical Spatiotemporally Destabilizes Mitochondrial Membranes for Cell Fate Manipulation

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Chemicals, Solvents and Materials

Reagents and solvents were purchased from Sigma-Aldrich Chemical Co. and used as received without further purification otherwise noted. N-isopropylacrylamide (NIPAAm) was purified by three recrystallizations in hexane and dried under vacuum. 2, 2'-azobis (isobutyronitrile) (AIBN, > 98%, Sigma) was recrystallized from ethanol. All polymerizations were conducted under nitrogen atmosphere. All amino acids and Wang resins were obtained from GL Biochem. (Shanghai) Ltd. MDA-MB-231 (human breast adenocarcinoma cells) was purchased from cell culture center of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). The Dulbecco's Modified Eagle Medium (DMEM) and Fetal Bovine Serum (FBS) were obtained from Wisent Inc. (Multicell, Wisent Inc., St. Bruno, Quebec, Canada). Penicillin/streptomycin were purchased from Invitrogen (Carlsbad, CA). β-actin antibody was purchased from Cell Signal Technology. MitoTracker Red was purchased from ThermoFisher scientific. Cell counting kit-8 assay (CCK-8) was purchased from Beyotime Institute of Biothechnology, China.

Characterizations

Spectroscopy

UV-Vis spectroscopy measurements were obtained on a Shimadzu 3600 UV/Vis spectrophotometer equipped with an S-1700 thermoelectric single cell holder. Fluorescence spectra were recorded using an F-280 fluorescence spectrophotometer, with excitation slit set at 5 nm, emission slit set at 5 nm, and PMT voltage set to 600 mV. ^{1}H NMR spectra were recorded in either $CDCl_3$ or DMSO-d_6 on a Bruker Avance III (400 MHz) spectrometer. Chemical shifts are expressed in parts per million (ppm), internally referenced relative to residual solvent peaks. Matrix-assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectra were recorded on a Microflex LRF Mass Spectrometer (Bruker Daltonics) under positive-ion mode using a solution of 2,5-Dihydroxybenzoic acid (20 mg mL^{-1} in acetonitrile/water/trifluoroacetic acid (70:30:0.1, v/v/v)) as the matrix. Samples were prepared by 1:1 (v/v) mixing of the sample solution and matrix solution. 1 μL of the mixture was then spotted onto a target plate and left to dry at room temperature prior to analysis.

Gel Permeation Chromatography (GPC)

GPC analyses were performed on a Shimadzu Gel Permeation Chromatography (GPC) equipped with Shimadzu RID-20A refractive index detector. DMF (HPLC grade, 0.4% LiBr) was used as the eluent at a flow rate of 1 mL min^{-1}. The system was calibrated with polystyrene standards. All samples were filtered with 0.22 μm filters prior to analysis.

Dynamic light scattering (DLS)

DLS measurements were performed on a Malvern Zetasizer Nano ZS. The instrument is equipped with a 4 mW He-Ne laser with a wavelength of 633 nm, an avalanche photodiode detector (APD) and a peltier system (2 – 90 ^{o}C). Scattered light were detected at an angle of 173°. The polydispersity index (PDI) which gives the width parameter of a distribution was derived from the standard deviation (σ) and Z-average size (Z_{avg}) of analysed samples (PDI= σ^2/Z_{avg}^2). A dilute solution of **anchor-PPC** was pipetted into a 1 mL disposable cuvette and kept at 37 ^{o}C. The sample was allowed to equilibrate for 20 min before measurements were taken in triplicate.

Scanning electron microscope (SEM)

SEM analysis of polymer aggregates was performed on a Nova Nano-SEM 430 + EDS system using an accelerating voltage of 10.0 kV, and a working distance of 5.0 mm. Samples were first incubated at 37 ^{o}C on a water bath in an Eppendorf tube for 30 min. 30 μL of incubated sample was then dropped onto a pre-warmed silicon wafer and left to dry at 37 ^{o}C on a heating block. Analysis of a population of 100 aggregates was performed using Image J, for the determination of average and standard deviation of diameters.

Cell Culture

The MDA-MB-231 breast cancer cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) that contains 10% FBS, and 1% penicillin/streptomycin at 37 ^{o}C in a humidified atmosphere containing 5% CO_2.

Confocal laser-scanning microscopy (CLSM)

CLSM analysis of **anchor-PPC** penetration into MDA-MB-231 cells, **anchor-PPC** *in situ* dehydration and aggregation on mitochondrial membranes and mitochondria depolarization were performed on a Zeiss LSM 710 microscope, equipped with a 63x oil-immersion objective and fitted monochromatic laser sources for fluorescence measurements. For **anchor-PPC** *in situ* dehydration and aggregation analysis, the cells were incubated at 37 ^{o}C using the pre-installed heating stage on the microscope and analysed after 15 min of 365 nm light irradiation (10 mW cm^{-2}). For the assessment of mitochondrial membrane potential, MDA-MB-231 cells (1×10^6) in a 35 mm confocal dish were treated with **anchor-PPC** (15 μM), followed by additional 2 h incubation for material penetration and targeting to mitochondra. Then, the cells were washed with pre-warmed PBS twice and additional 3 × 15 min incubation in serum free medium at 37 ^{o}C. After photo irradiation by 365 nm light (10 mW cm^{-2}, 15 min), the cells were washed with PBS, and added a serum-free medium containing JC-1 dye, subjected to additional 20 min incubation at 37 °C. Finally, the cells were washed with cold JC-1 buffer solution two times before visualized of the green and red fluorescence levels using a Zeiss LSM 710 microscope. The images were obtained at 488 nm

excitation and 530 nm emission for green (JC-1 monomers) and at 543 nm excitation and 590 nm emission for red fluorescence (JC-1 aggregates).

Western Blots

Cells were seeded at a density of 5,000 to 10,000 cells/cm^2 24 h prior to treatment. Then, cells were treated with 15 μM of **anchor-PPC** and irradiation with 365 nm light (10 mW cm^{-2}) for 15 min, after which the cells were incubated for additional 12 h. Afterwards, cells were washed twice with cold PBS and scraped in PBS containing proteinase inhibitors. Cells were centrifuged at 300 rpm and washed once in cold PBS-containing inhibitors. Cells were lysed for 30 min at 4 °C in cold PBS containing 1 % Triton X-100 and inhibitors. Lysates were cleared by centrifugation at 20,000 g for 10 min and protein concentrations were determined by BCA assays (Pierce). Samples were adjusted to 2 mg/ml in reducing LDS sample buffer (Invitrogen) containing 0.05 % β-mercaptoethanol and boiled for 3 min. Samples were subjected to 10 % SDS-PAGE and blotted onto a PVDF-FL membrane (Millipore) using the wet blot protocol from Biorad. The membrane was blocked with PBS containing 1x casein blocking buffer (Sigma) and primary antibodies were incubated in PBS casein buffer containing 0.1 % Tween-20 overnight at 4 °C. Membranes were washed three times in PBS containing 0.1% Tween-20 and secondary antibodies were incubated for 2 h at room temperature. Membranes were washed 3 times in PBS 0.1% Tween-20 and signals were visualized by chemiluminescence on a Typhoon Trio Variable Mode Imager. Band density was calculated using NIH Image J software.

CCK-8 Test

In order to check cell viability, cell counting kit-8 was used. MDA-MB-231 cells were incubated with 15 μM of **anchor-PPC** for 2 h and subjected to 365 nm light (10 mW cm^{-2}) for 15 min, after which the cells were incubated for additional 12 h. Cell suspension in a 96-well plate was incubated with 10 μL of the CCK-8 solution for each well for 2.5 h at 12 h after 365-nm light application. Then, absorbance of each sample was measured at 450 nm.

Immunostaining for active caspase-3

In the spatiotemporal manipulation experiments, the **anchor-PPC**-treated/light-irradiated cells and KLAK-treated cells were washed with PBS and fixed with 4% paraformaldehyde. Fixed cells were permeabilized with 50% and 100% ice-cold methanol for 30 min at -20 °C. Then, these cells were washed with PBST. PBST containing 5% FBS and 2 mg mL^{-1} BSA was used for 2 h at room temperature to block the samples. Then, primary antibody for activated caspase-3 was added to cells and samples were incubated overnight at 4 oC. After washing the cells with PBST 3 times for 15 min at room temperature, Cy5-labeled secondary antibody was added to cells for 2 h at room temperature. Cells were washed with PBST and placed into serial diluted glycerol solution from 100% to 50% for confocal microscopy observation.

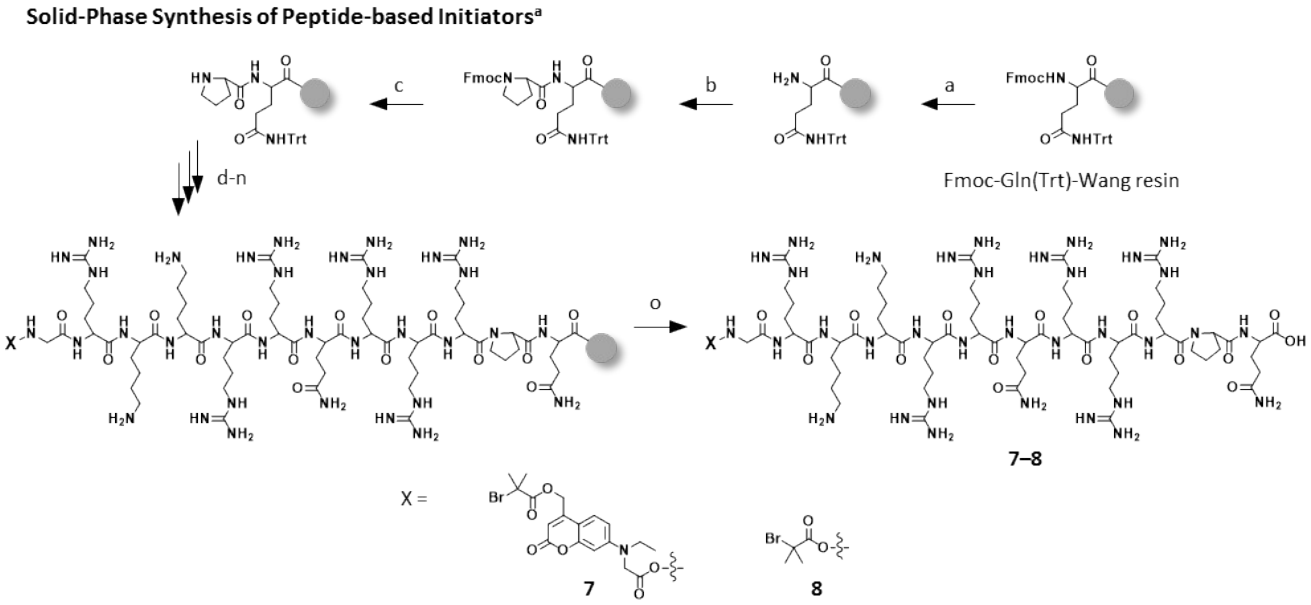
3D growth assay

3D growth assay was performed by using the multicellular cancer spheroids (MCCSs). MCCSs from breast cancer cell line MDA-MB-231 were generated using the liquid overlay method in a 96-well plate. Briefly, serum free DMEM medium with 1.5% (w/v) agar was prepared and sterilized. 50 μL of the agar solution was added to the bottom of each well of the 96 well plates to prevent cell adhesion onto the well surface. Plates were allowed to cool down for 45 min before use. MDA-MB-231 cells were trypsinized, counted and then seeded at the density of 600 cells/well. Plates were centrifuged for 15 min at 1500 rcf at room temperature. When the spheroids are formed and dense (after 3 days), they were treated with **anchor-PPC** in the absence or presence of 365 nm light to assess its capability of phase transition and disruption of mitochondrial membranes. For the 3D growth assay, after the indicated treatment, the medium was refreshed every 2 days, and the tumor size, the released cytochrome c and the activated caspase-3 were analyzed after 6 days.

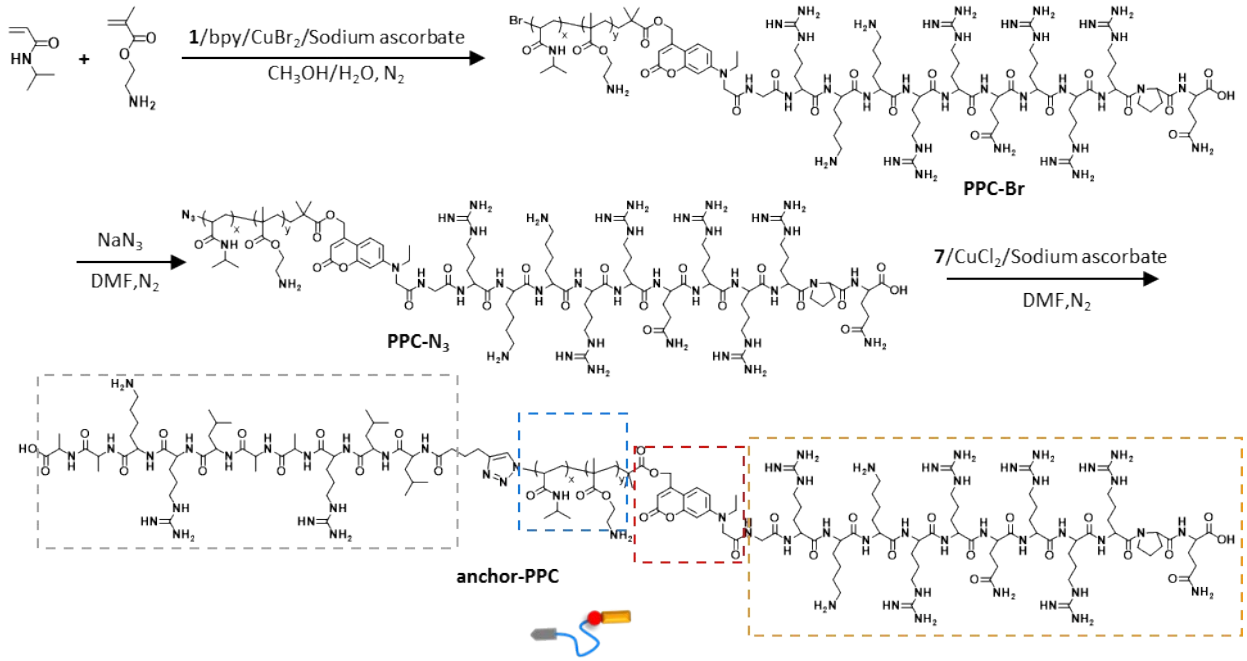
Statistical analysis

All data were presented as means ± standard deviation (s.d.) unless indicated otherwise. The statistical analysis for two-group comparisons was performed by using Student's t-test for normally distributed samples with equal variance. Significance was established at the $p \leq 0.05$; 0.01 and 0.001 level, as indicated in the figures. Not significant represented as n.s..

Experimental



Scheme S1. Solid-phase synthesis of peptide-based innitiators. Reagents and conditions: (a) 20% piperidine in DMF; (b) Fmoc-Pro-COOH, HBTU, 0.4 M N-Methyl morpholine in DMF; (c) 20% piperidine in DMF; (d-f) i. Fmoc-Arg(pbf)-OH, HBTU, 0.4 M N-Methyl morpholine in DMF; ii. 20% piperidine in DMF; (g) i. Fmoc-Gln(Trt)-OH, HBTU, 0.4 M N-Methyl morpholine in DMF; ii. 20% piperidine in DMF; (h-i) i. Fmoc-Arg(pbf)-OH, HBTU, 0.4 M N-Methyl morpholine in DMF; ii. 20% piperidine in DMF; (j-k) i. Fmoc-Lys(Boc)-OH, HBTU, 0.4 M N-Methyl morpholine in DMF; ii. 20% piperidine in DMF; (l) i. Fmoc-Arg(pbf)-OH, HBTU, 0.4 M N-Methyl morpholine in DMF; ii. 20% piperidine in DMF; (m) i. Fmoc-Gly-OH, HBTU, 0.4 M N-Methyl morpholine in DMF; ii. 20% piperidine in DMF; (n) 6 or 2-Bromoisobutyric acid, HBTU, 0.4 M N-Methyl morpholine in DMF; (o) TFA/TIS/H2O (v/v/v = 95:2.5:2.5), 3 h



Scheme S2. Schematic illustration of peptide-based initiator imitated ATRP polymerization and **anchor-PPC** synthesis.

Synthesis of photolabile linker

Compound **1**: A solution of 3-aminophenol (10 g, 92.5 mmol) and K_2CO_3 (12.5 g, 92.5 mmol) in DMF (15 mL) was stirred at 25 °C for 15 min. Iodoethane (14 g, 92.5 mmol) was then added and heated at 100 °C for 2 h. The reaction mixture was cooled to 25 °C and filtered to remove solid impurities. Water (30 mL) was added to the filtrate and extracted with EtOAc (20 mL × 3), then the combined organic layer was concentrated under reduced pressure, brown oil appeared. Purification with column chromatography (petroleum ether: ethyl acetate = 5:1) gave 5.9 g (45 mmol, 48 % yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ=7.03 (t, J=8.1 Hz, 1H, ArH), 6.21 (t, J=9Hz, 2H, ArH), 6.13 (s, 1H, ArH), 3.14 (q, J=7.5Hz, 2H, -CH_2-), 1.26 (t, J=7Hz, 3H, -CH_3); MS(ESI): 136.1 $[M-H]^+$.

Compound **2**: A mixture of **1** (3.1 g, 22.6 mmol), ethyl acetoacetate (3.2 mL, 24.9 mmol), and $BiCl_3$ (0.72 g, 2.3 mmol) was stirred at 75 °C for 2 days. The reaction mixture was then cooled to room temperature, diluted with dichloromethane and filtered. The obtained filtrate was evaporated under vacuum to yield brown oil. Purification with column chromatography (petroleum ether: ethyl acetate = 5:1) gave 1.5 g (7.5 mmol, 33 % yield) of 2 as a yellowish solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=7.38-7.36 (d, J=8.7Hz, 1H, ArH), 6.53-6.50 (d, J=9Hz, 1H, ArH), 6.47-6.46 (d, J=1Hz, 1H, ArH), 6.0 (s, 1H, ArH), 3.26-3.23 (t, J=6Hz, 2H, -CH_2-), 2.37-2.36 (s, 3H, -CH_3), 1.34-1.30(t, J=6Hz, 3H, -CH_3); MS(ESI):204.1 $[M+H]^+$.

Compound **3** A solution of **2** (0.5 g, 2.5 mmol), tert-butyl bromoacetate (0.6 mL, 3.8 mmol), K_2CO_3 (1.0 g, 7.5 mmol), KI (38.0 mg, 0.25 mmol), and tetrabutylammonium bromide (81.0 mg, 0.25 mmol) in CH_3CN (20 mL) was refluxed for 3 days. The reaction mixture was allowed to cool to room temperature. After filtration, the solvent was removed under vacuum. The residue was then dissolved in CH_2Cl_2 and washed with H_2O (3 × 50 mL). The organic layer was then dried over Na_2SO_4 and evaporated to yield the crude product. Purification with column chromatography (petroleum ether: ethyl acetate = 5:1) gave 0.63 g (2.0 mmol, 80 % yield) of **3** as yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=7.43-7.41 (d, J=9Hz, 1H, ArH), 6.59-6.56 (d, J=9Hz, 1H, ArH), 6.49 (s, 1H, ArH), 6.00 (s, 1H, RC=CH), 3.99 (s, 2H, -CH_2-), 3.56-3.50 (q, J=5.7Hz, 2H, -CH_2-), 2.37 (s, 3H, -CH_3), 1.48 (s, 9H, -CH_3), 1.29-1.25 (t, J=7.2Hz, 3H, -CH_3); MS(ESI): 318.3 $[M+H]^+$.

Compound **4**: SeO_2 (0.43 g, 2.8 mmol) and **3** (0.6 g, 1.9 mmol) were suspended in 50 mL p-xylene, the reaction mixture was refluxed under vigorous stirring with the protection of an argon atmosphere. After 24 h, the mixture was filtered and concentrated under reduced pressure. The dark brown residual oil was dissolved in methanol (50 mL), then sodium borohydride (0.14 g, 3.8 mmol) was added, and the solution was stirred for 3h at room temperature. Thereafter, the suspension was carefully neutralized with 1 M HCl, diluted with H_2O, and partially concentrated under reduced pressure to remove methanol. The mixture was extracted with CH_2Cl_2 and the obtained organic phase was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuum. The obtained oil was purified by column chromatography (petroleum ether: ethyl acetate = 5:2) to yield 0.40 g (1.27 mmol, 67 %) of **4** as a brown solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=7.30 (d, J=9.5Hz, 1H, ArH), 6.50 (dd, J=9Hz, 2H, ArH), 6.31 (s, 1H, RC=CH), 4.85 (d, J=3Hz, 2H, -CH_2-), 3.97 (s, 2H, -CH_2-), 3.50 (q, J=7Hz, 2H, -CH_2-), 1.47 (s, 9H, -CH_3), 1.24 (t, J=9Hz, 3H, -CH_3). MS(ESI): 333.8 $[M]^+$.

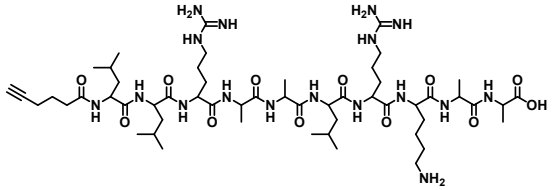
Compound **5**: In dark condition, to a solution of tert-butyl ester **5** (300mg, 0.46 mmol) in CH_2Cl_2 (3ml) was added TFA (5 ml) dropwise in ice-bath. The reaction mixture was stirred for 4 h at room temperature (in dark condition). After removing the solvent, TFA and by-products under vacuum, the obtained yellow solid was then washed by water several times and dried under vacuum. The gained compound was directly used in the next step without purification. MS(ESI): 276.1 $[M-H]^+$.

Compound **6**: In dark condition, **d** (500 mg, 1.59 mmol) and 243 mg (2.39 mmol) of triethylamine were dissolved in 6-7 mL of toluene. Over ice, and under nitrogen, 2-brommoisobutyryl bromide (550 mg, 2.39 mmol) was added dropwise. The reaction mixture was left to react overnight before filtering. The solid was disregarded and the filtrate was washed with 0.1 M sodium carbonate (3 x 100 mL) and di-water (3 × 100 mL) before being dried over magnesium sulfate. The toluene was removed under reduced pressure and the brown liquid was purified using flash chromatography (dichloromethane: ethyl acetate = 4:1) to yield a yellow oil (Yield 63%). MS(ESI): 482.4 $[M+H]^+$.

Synthesis of peptide-based initiators

Compound **7** and **8**: Peptide initiators were synthesized by standard 9-fluorenylmethoxycarbonyl (Fmoc) solid-phase method on a 0.4 mmol scale. Wang-resin was used as a polymeric support. The Fmoc groups on the resin were removed by a DMF/piperidine (80/20) mixture for 15-20 min at room temperature. Amino acids (10 equiv.) were then consecutively coupled to the de-protected Wang-resin using NMM (0.4 M) and HBTU (10 equiv.) as the coupling agents in anhydrous DMF at room temperature for 1 h. Fmoc de-protection and completion of each coupling reaction were confirmed by qualitative ninhydrin test (ninhydrin, phenol, VC, 1:1:1, v/v/v). Cycling the de-protection and coupling procedures mentioned above. Finally, either compound **6** or 2-Bromoisobutyric Acid was coupled to the peptide terminal to obtain compound **7** and **8**. Deprotection and cleavage of peptides from the resin was performed by using the mixture of Trifluoroacetic acid (95%, v/v), H_2O (2.5%, v/v), and Triisopropylsilane (2.5%, v/v) for 2.5 h in an ice-bath. Each peptide was precipitated into cold anhydrous diethyl ether, dried under vacuum, and characterized by MALDI-TOF and HPLC.

Synthesis of peptide anchor-N_3



The alkyne-functionalized anchor peptide LLRAALRKAA (anchor-N_3) was synthesized by standard solid-phase peptide synthesis method at a 0.4 mmol scale. Wang-resin was used as a polymeric support. The Fmoc groups on the resin were removed by a DMF/piperidine (80/20) mixture for 15-20 min at room temperature. Amino acids (10 equiv.) were then consecutively coupled to the de-protected Wang-resin using NMM (0.4 M) and HBTU (10 equiv.) as the coupling agents in anhydrous DMF at room temperature for 1 h. Fmoc de-protection and completion of each coupling reaction were confirmed by qualitative ninhydrin test (ninhydrin, phenol, VC, 1:1:1, v/v/v). Cycling the de-protection and coupling procedures mentioned above. Finally, 5-Hexynoic acid was coupled to the peptide terminal to obtain anchor-N_3. Deprotection and cleavage of peptides from the resin was performed by using the mixture of Trifluoroacetic acid (95%, v/v), H_2O (2.5%, v/v), and Triisopropylsilane (2.5%, v/v) for 2.5 h in an ice-bath. The peptide was precipitated into cold anhydrous diethyl ether, dried under vacuum, and characterized by MALDI-TOF and HPLC.

Synthesis of anchor-PPC and anchor-PPC-ctrl

Both **anchor-PPC** and **anchor-PPC-ctrl** was synthesized *via* sequential atom transfer radical polymerization (ATRP) polymerization and azide-alkyne click reaction. Firstly, the alkyne-terminated PPC (PPC-N_3, see scheme S2) or PPC-ctrl (PPC-ctrl-N_3) was prepared. In a typical experiment, NIPAAm (520.5 mg, 4.6 mmol), 2-Aminoethyl methacrylate (51.7 mg, 0.4 mmol), and initiator **7** (81.2 mg, 0.04 mmol) were added to a 200-ml round bottom flask equipped with a magnetic stirring bar and dissolved with 50 ml CH_3OH/H_2O. The mixture was purged with nitrogen for at least 30 min. A solution of catalyst was prepared by adding $CuBr_2$ (13.4 mg, 0.06 mmol), bipyridyl (bpy) (49.0 mg, 0.12 mmol), and sodium ascorbate (11.9 mg, 0.06 mmol) to a 10-mL flask equipped with magnetic stirring bar, dissolved with 4 ml CH_3OH/H_2O, purged with nitrogen. Transfer the catalyst solution to the 200-ml round bottom flask reactor under the protection of nitrogen, sealed with rubber septum and started ATRP polymerization at room temperature. All through the reaction, the mixture was protected by nitrogen. After a 12-h reaction, the reaction was stopped by exposure to oxygen. The obtained polymer was passed through a column of neutral alumina, and then rotary evaporated to remove the methanol, dialyzed against water (MWCO = 10 kDa) and finally lyophilized for recovery to obtain PPC-Br. After the purification, the obtained PPC-Br was dissolved in DMF, NaN_3 (2.5 times excess to the mole of bromo group) was added, causing the bromine chain end to be substituted by an azide by means of the copper−bipyridyl catalyst under the protection of nitrogen.[1] The reaction mixture was left for 16 h to ensure complete substitution, after which the polymer was filtered through a column filled with neutral alumina to remove the copper complex before the polymer was dialyzed against water (MWCO = 10 kDa) and finally lyophilized for recovery to obtain PPC-N_3 (Yield 90.5%). The molecular weight of the polymers was determined by GPC (Mn= 32.1 kD, PDI = 1.23). Similarly, the PPC-ctrl-N_3 (Yield 92.1%) was synthesized by the same protocol. The molecular weight of the polymers was determined by GPC (Mn= 32.6 kD, PDI = 1.21).

The click coupling reaction between anchor-N_3 and alkyne-functionalized PPC/PPC-ctrl was performed by Cu(I) catalysis in a 10 ml Schlenk flask with DMF as solvent and $CuCl_2$/sodium ascorbate as catalyst. After 10 h, the polymer solution was exposed to air, diluted with THF, and passed through neutral alumina to remove the copper catalyst. The solvent was removed by rotary evaporation, dialyzed against water (MWCO = 10 kDa) and finally lyophilized for recovery. The molecular weight of the polymers was determined by GPC. **anchor-PPC** (Yield 95.7%): Mn= 35.5 kD, PDI = 1.23; **anchor-PPC-ctrl** (Yield 94.8%): Mn= 36.0 kD, PDI = 1.25.

Supplementary Figures

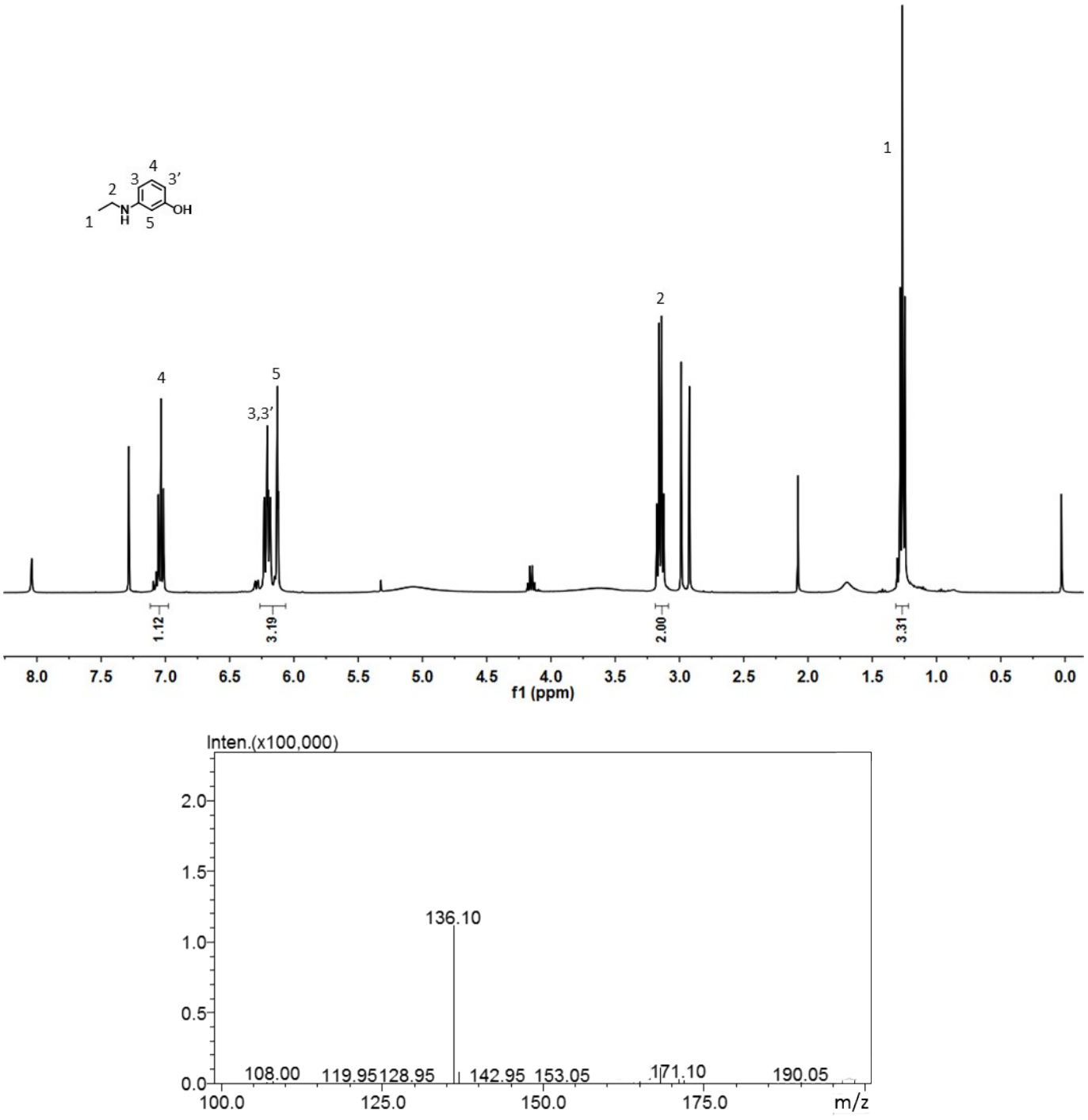


Figure S1. ^{1}H NMR and ESI-MS spectrum of compound **1**.

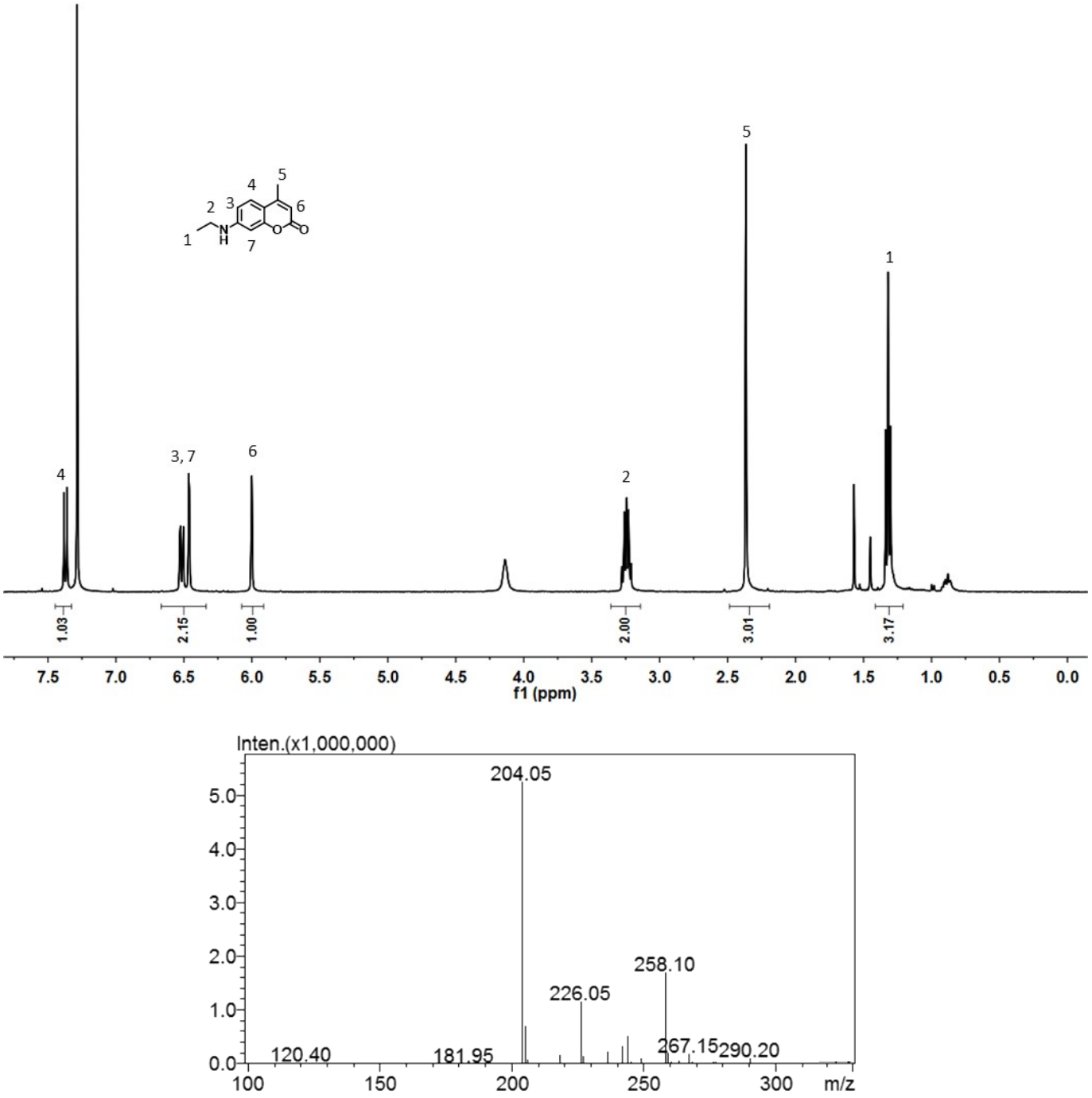


Figure S2. [1]H NMR and ESI-MS spectrum of compound **2**.

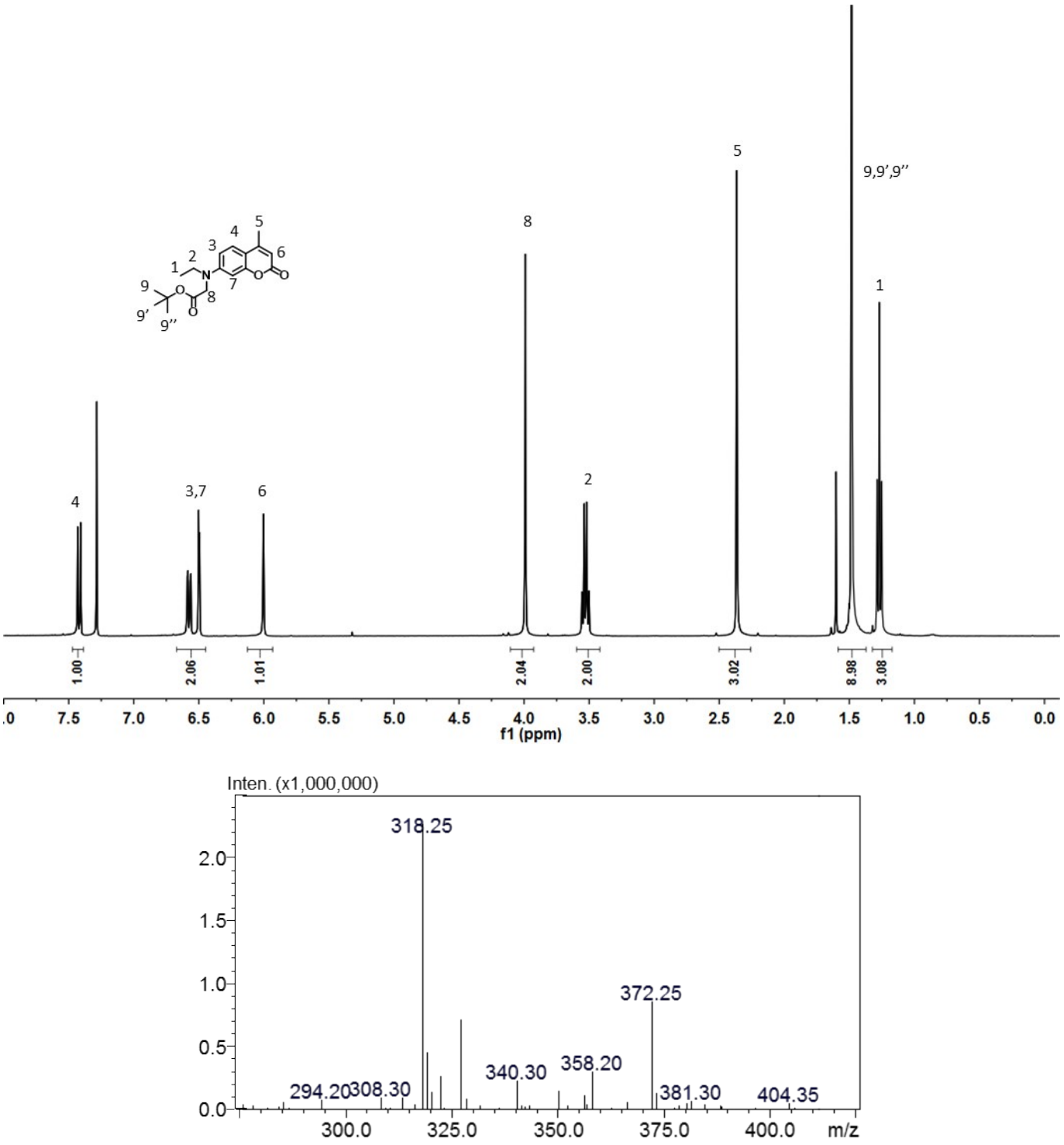


Figure S3. [1]H NMR and ESI-MS spectrum of compound **3**.

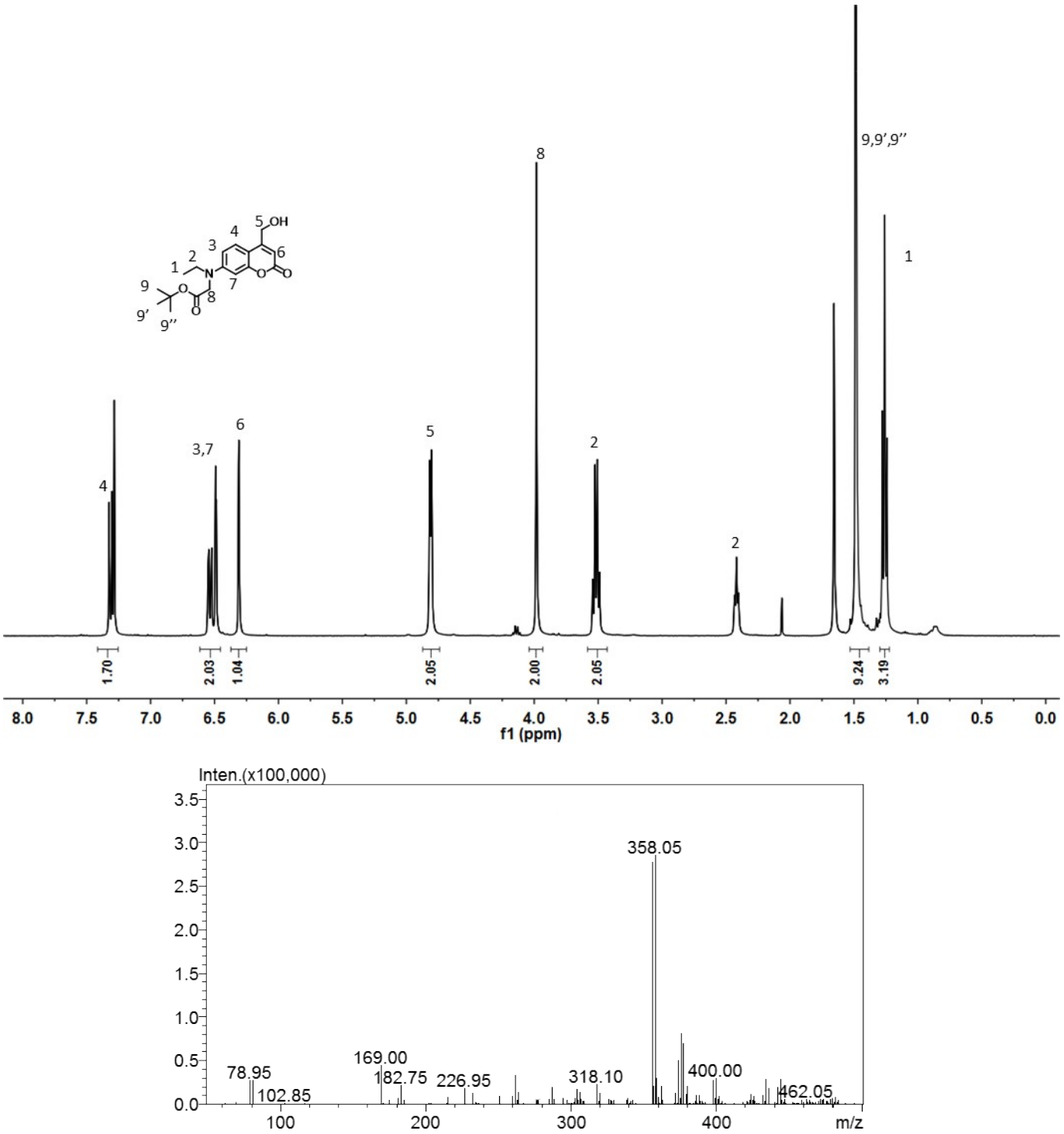


Figure S4. [1]H NMR and ESI-MS spectrum of compound **4**.

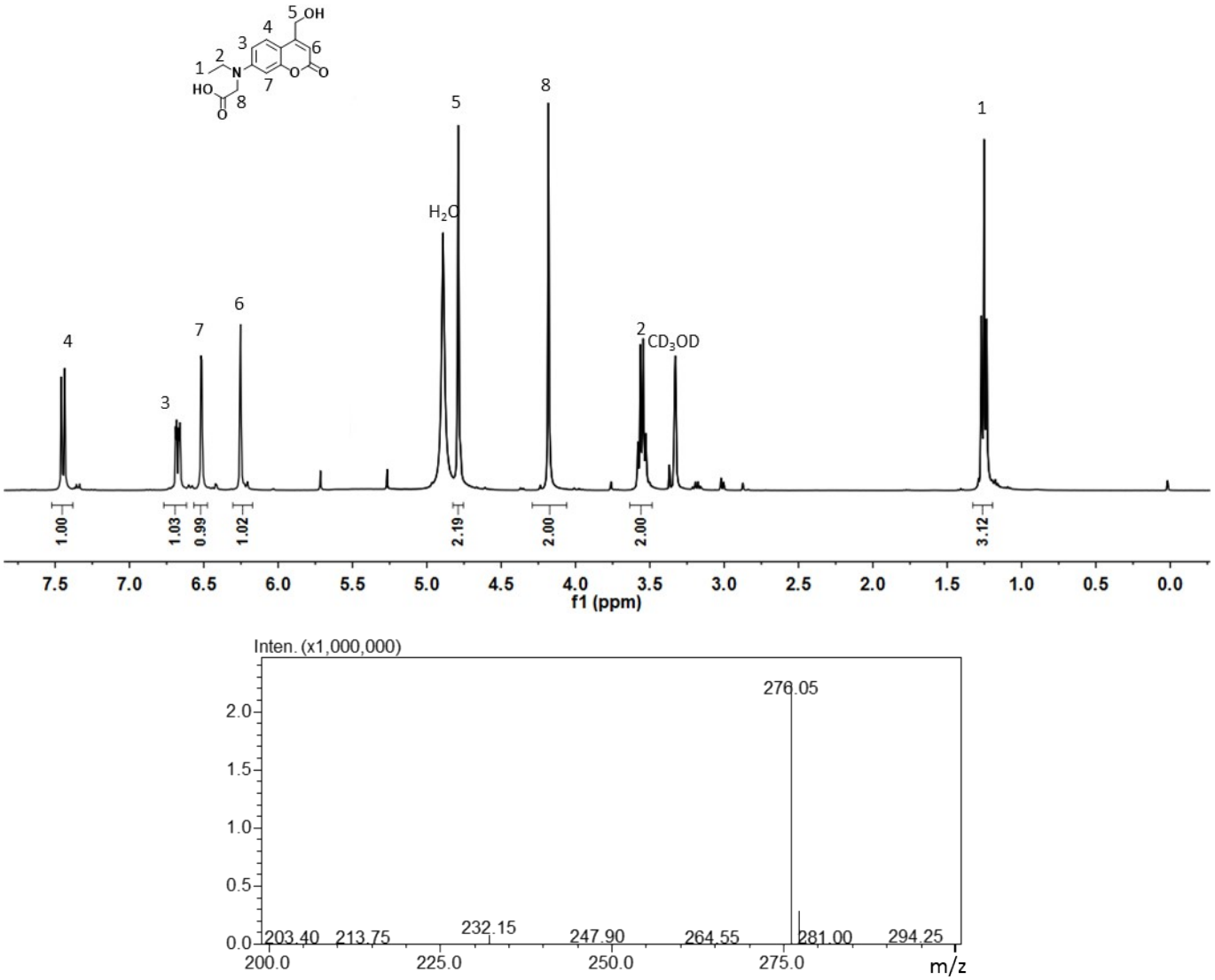
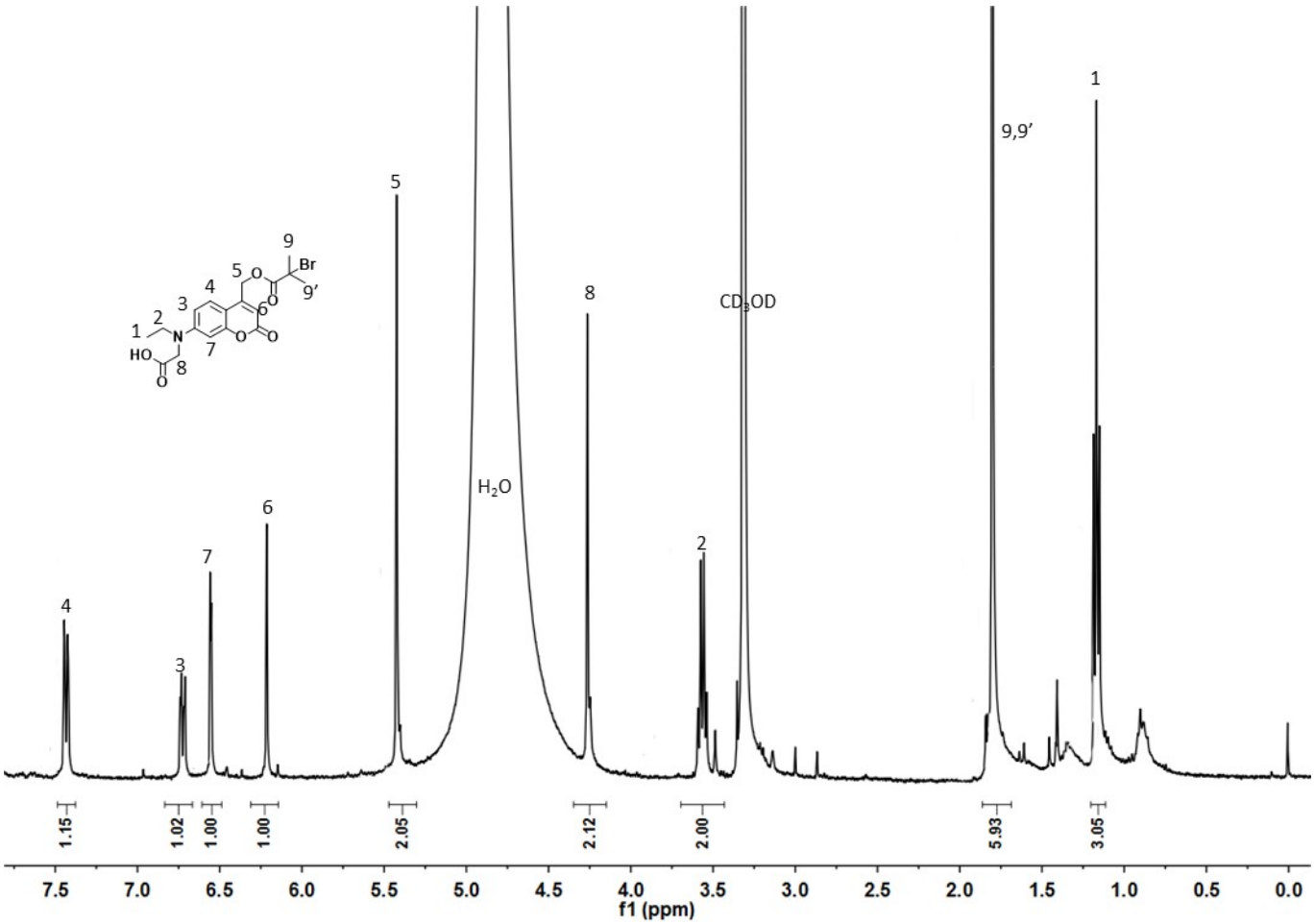


Figure S5. ^{1}H NMR and ESI-MS spectrum of compound **5**.



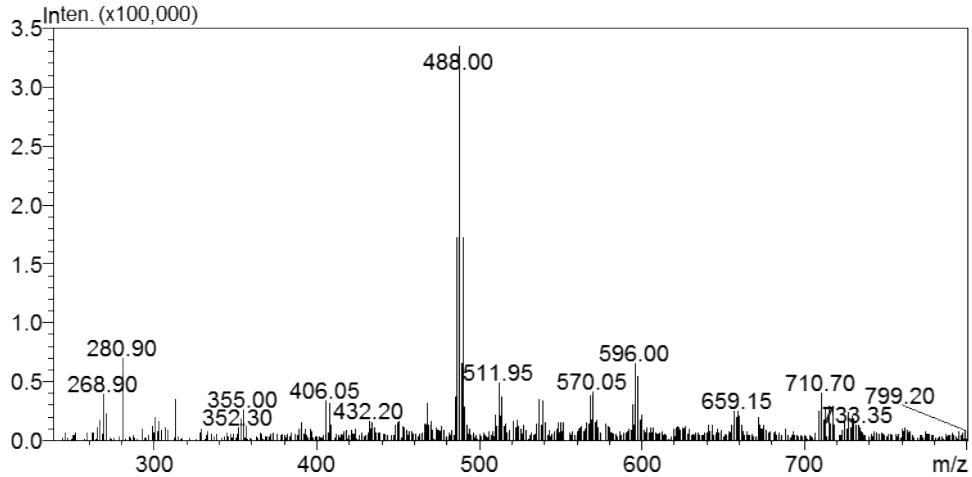


Figure S6. ^{1}H NMR and ESI-MS spectrum of compound **6**.

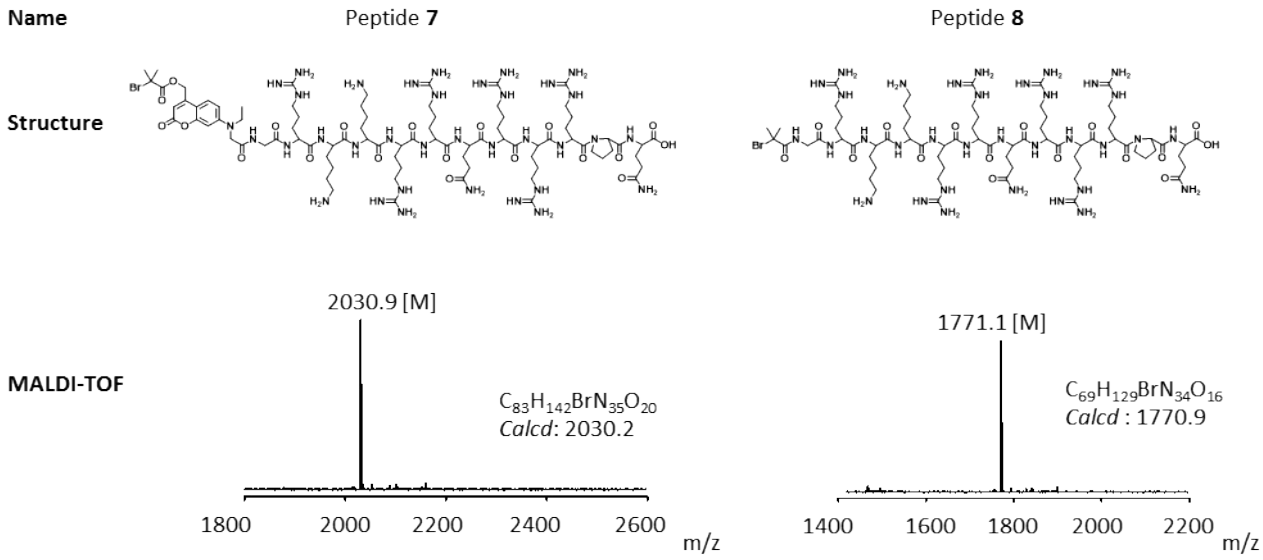


Figure S7. MALDI-TOF spectrum of compound **7** and **8**.

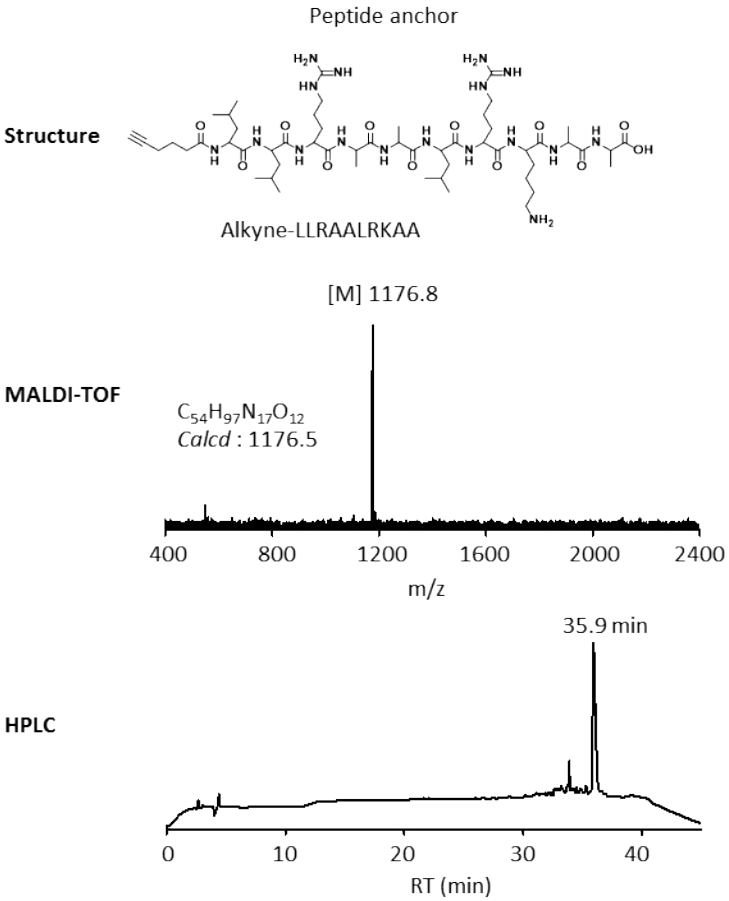


Figure S8. MALDI-TOF and HPLC spectrum of mitochondrial anchor peptide anchor-N_3.

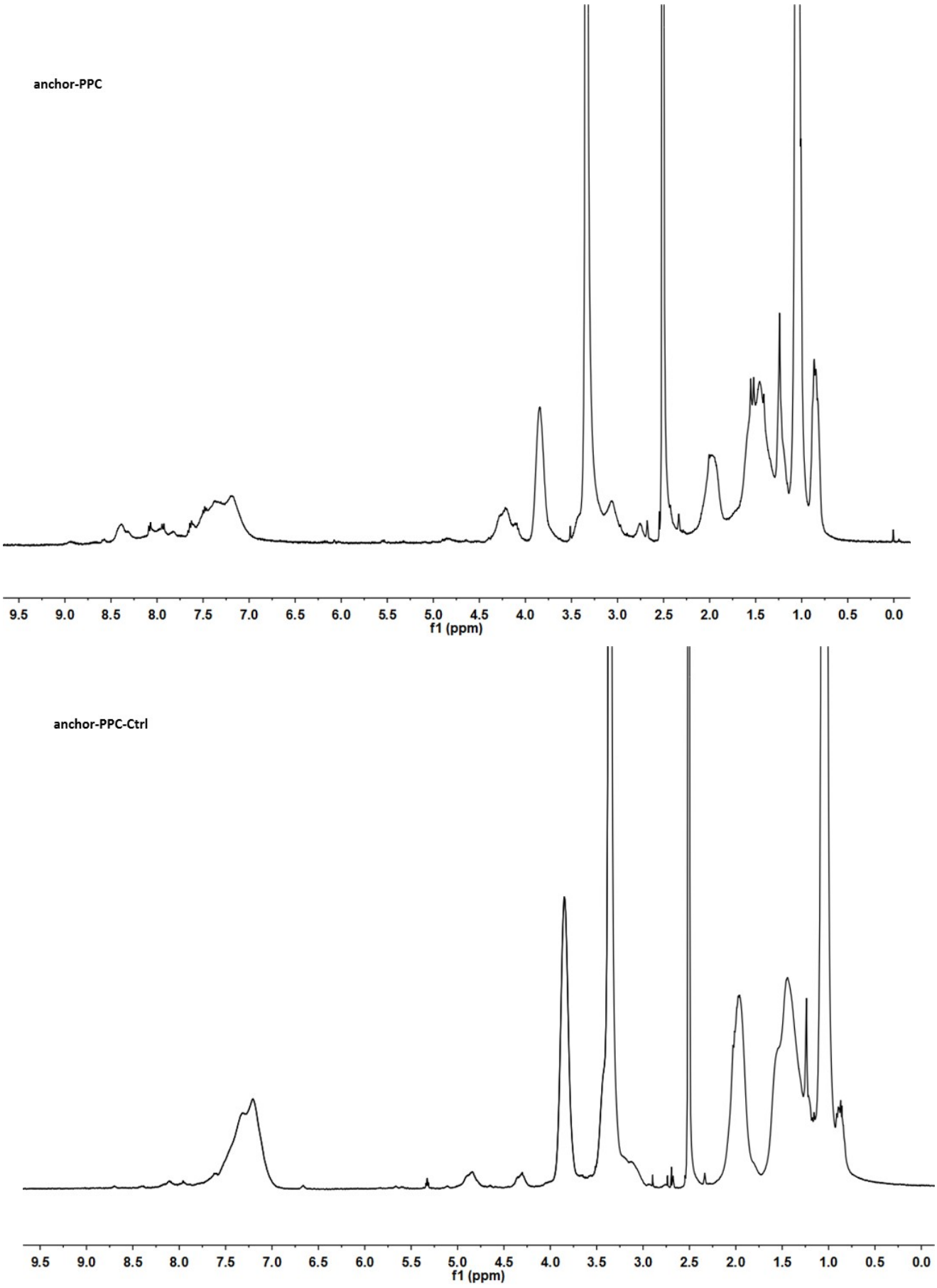


Figure S9. ^{1}H NMR spectrum of **anchor-PPC** and **anchor-PPC-Ctrl**.

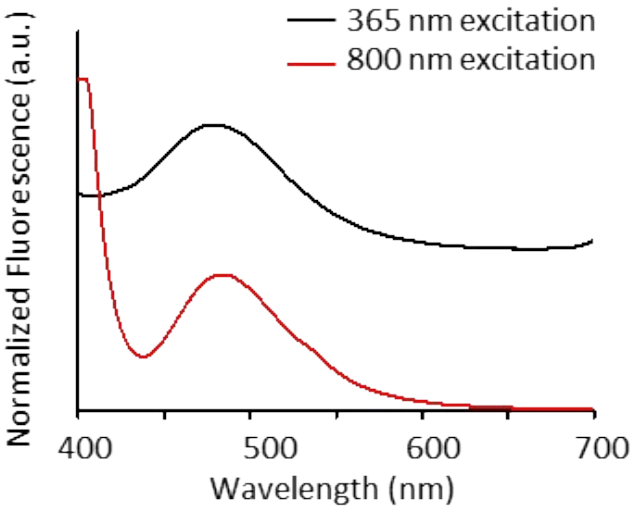


Figure S10. Emission spectra of compound **6** in water (upper: single photon excitation at 365 nm, lower: two photon excitation at 800 nm).

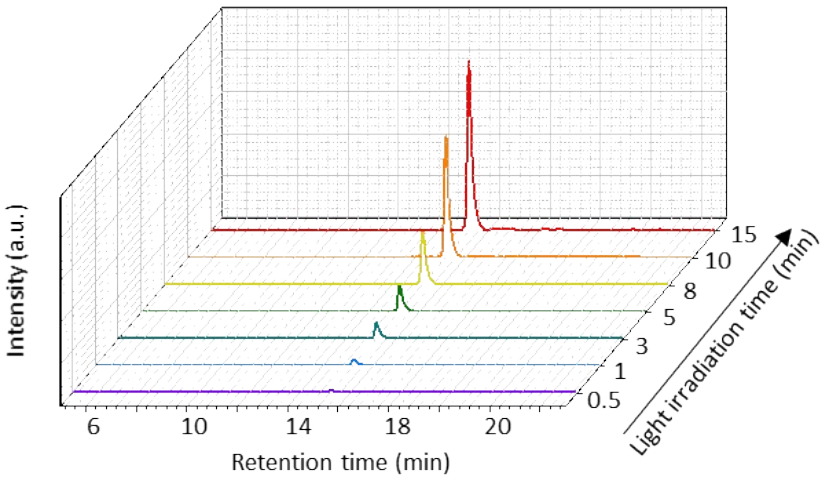


Figure S11. Time-dependent removement of hydrophilic peptide on **anchor-PPC**. HPLC detection of the removed hydrophilic peptide at different time point.

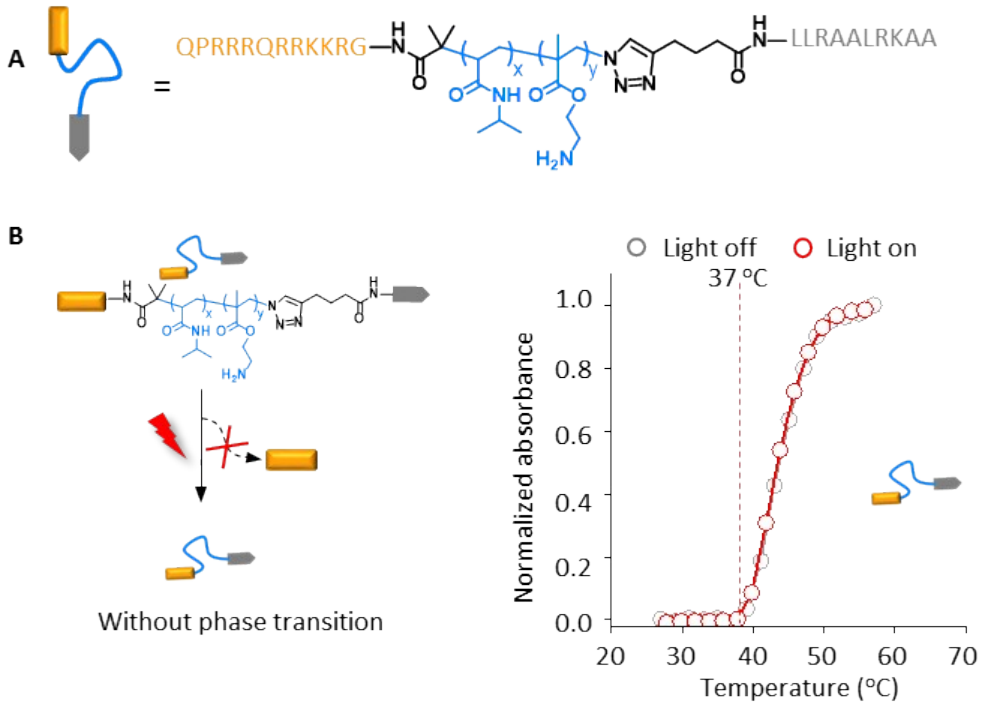


Figure S12. Chacracterizaiton of **anchor-PPC-ctrl.** A) Structure of photostable **anchor-PPC-ctrl**. B) The phase transiton temperature of **anchor-PPC-ctrl** (15 μM) has negligible change due to the hydrophilic peptide on **anchor-PPC-ctrl** cannot be removed upon light (365 nm, 10 mW cm^{-2}) irradiation.

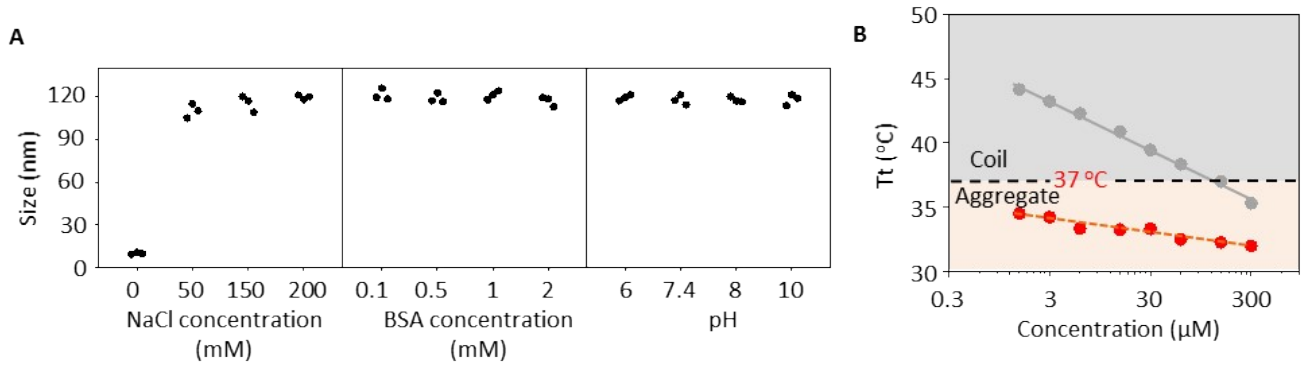


Figure S13. Influence of A) salts, proteins, pH and B) concentration on the aggregation behavior of **anchor-PPC**.

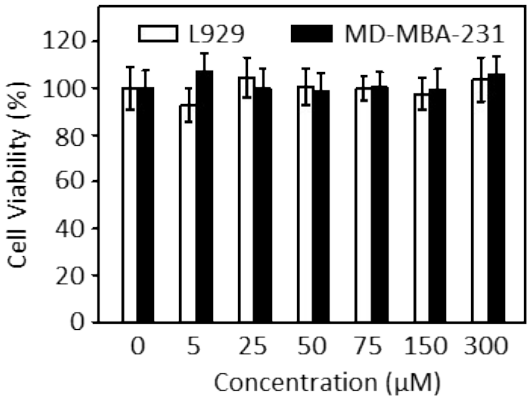


Figure S14. Cell viability assay. L929 or MDA-MB-231 cells were treated with different concentration of **anchor-PPC** and cultured for a period of 72 h**.** Error bars indicated s.d. (n = 3).

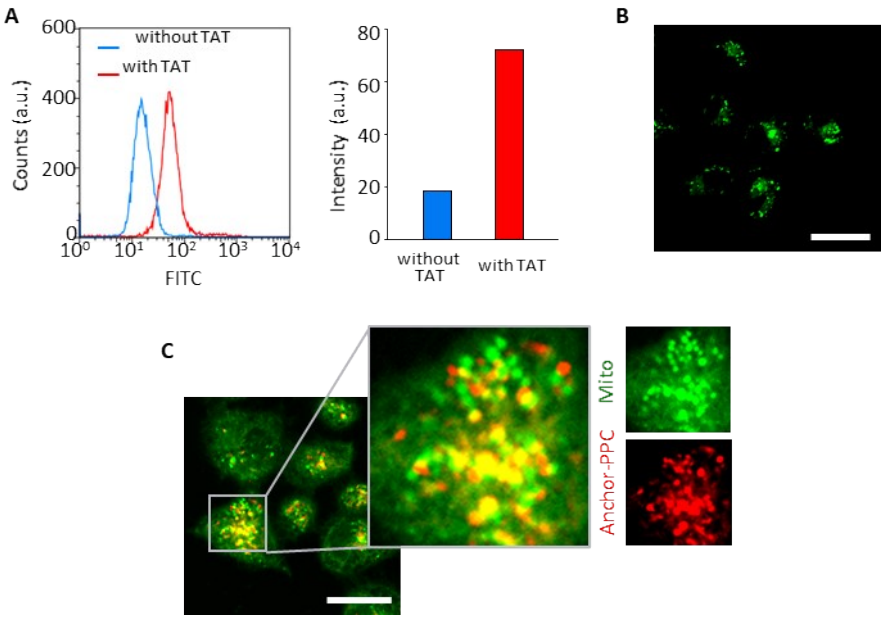


Figure S15. Anchor-PPC penetrates into MDA-MB-231 cells and targets to mitochondria. (A) FACS confirmation of TAT peptide aids PPC penetrating into cells. B) PPC that without peptide anchor preferred diffuse away. No apparent Cy5 labeled PPC colocalized with mitochondria. Mitochondria, *green*. *Scale bars*, 35 μm. C) Colocalization of **Anchor-PPC**$_{\mathbf{Cy5}}$ and mitochondria. *Scale bars*, 20 μm.

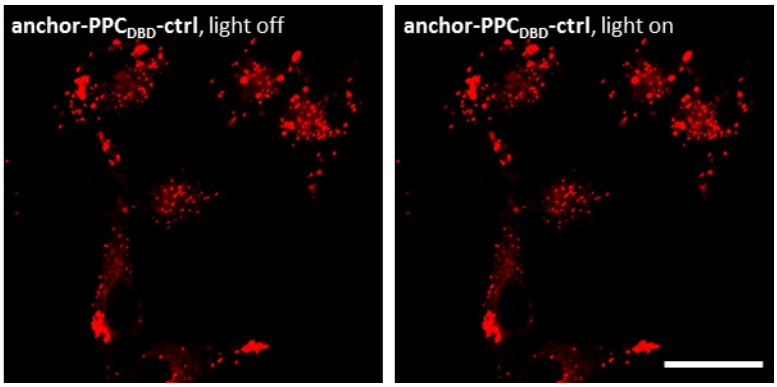


Figure S16. Photostable **anchor-PPC$_{DBD}$-ctrl** cannot dehydrate and aggregate at body temperature. There is no detectable DBD green fluorescence after 365 nm light (10 mW cm^{-2}, 15 min) irradiation. The red fluorescence is originated from Mitotracker red. *Scale bars*, 35 μm.

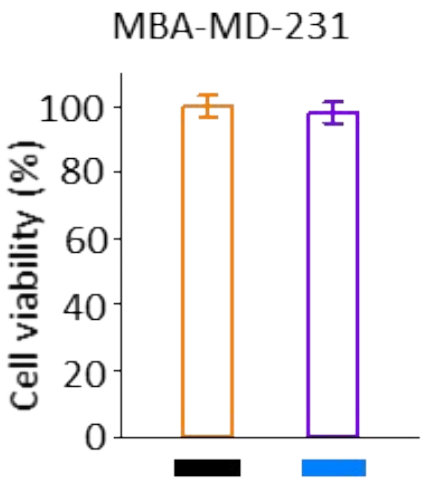


Figure S17. Phototoxicity measurement of 365 nm light to MDA-MB-231 cells. Cell viability was determined by CCK-8 assay after 15 min exposure of MDA-MB-231 cells to 365 nm light (10 mW cm^{-2}) and further incubation for 12 h. Error bars indicated s.d. (n = 3).

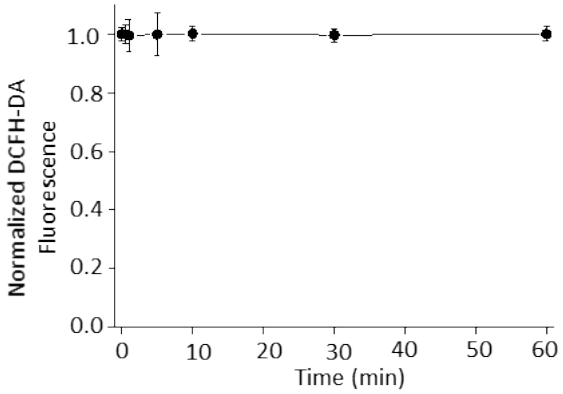


Figure S18. Time dependent ROS detection. Error bars indicated s.d. (n = 3).

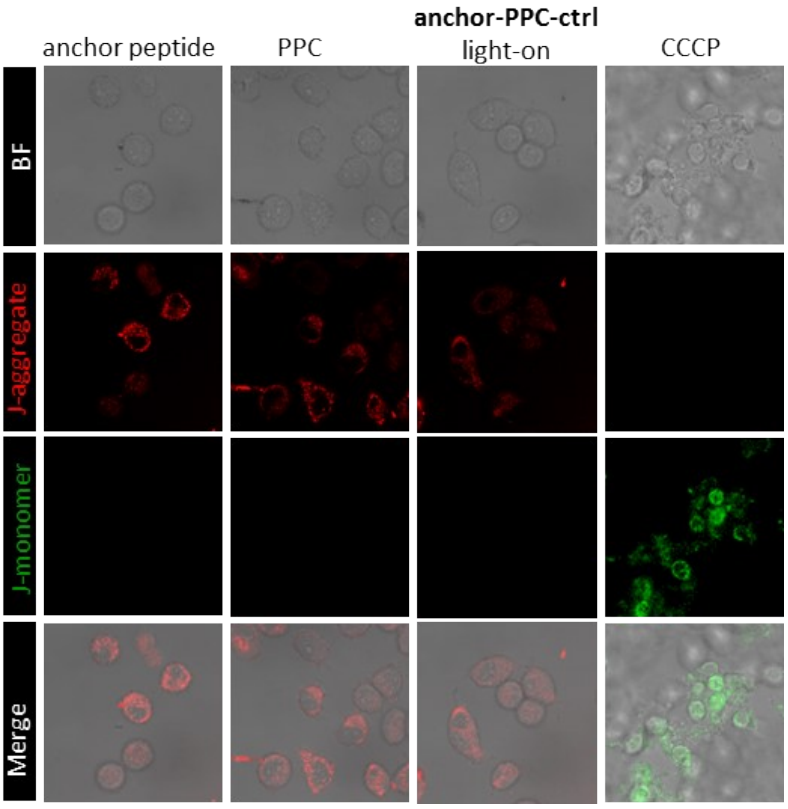


Figure S19. Detection of mitochondrial membrane potential changes in MDA-MB-231 cells. JC-1 forming red-emitting aggregates in the mitochondrial matrix, green-emitting monomers in the cytoplasm. Increase of green fluorescence suggests loss of mitochondrial membrane potential. CCCP (carbonylcyanide-m-chlorophenyl hydrazone) is a control apoptosis inducer that depolarizes mitochondrial membranes. Negligible mitochondria membrane damage was observed in anchor peptide, PPC polymer backbone, and **anchor-PPC-ctrl**/light-on treated groups.

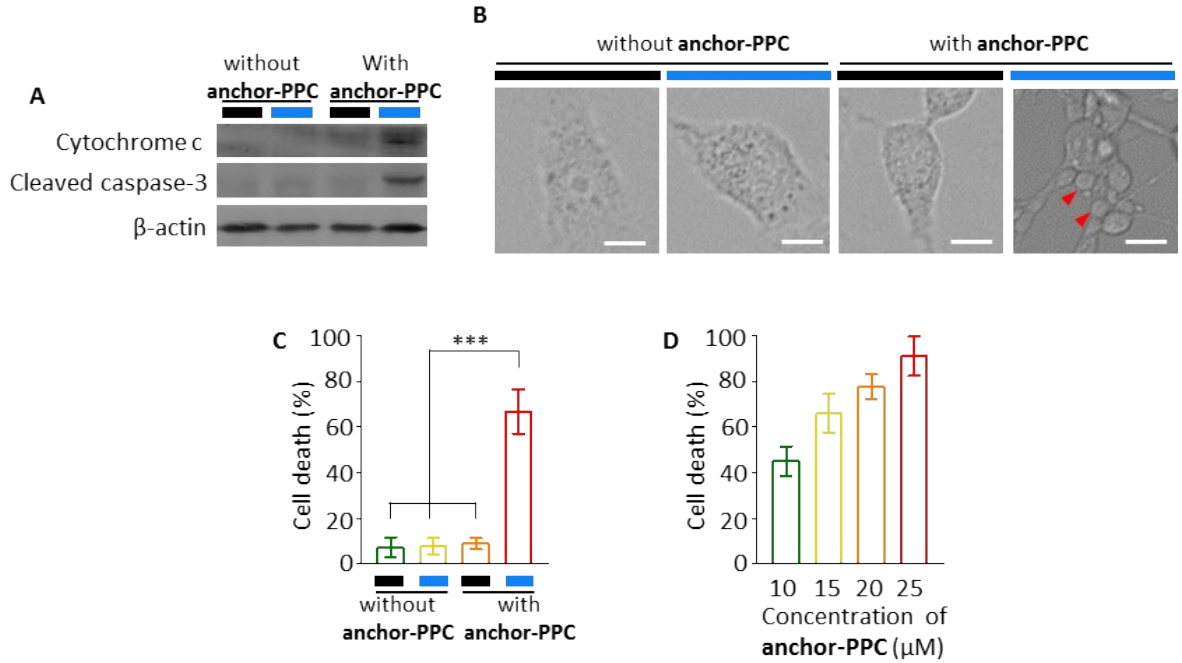


Figure S20. Mitochondrial membrane disruption leads to cytochrome c release and induces caspase-dependent apoptosis. A) Western blot measurement the changes of cytosolic cytochrome c and cleaved caspase-3 with the indicated treatment. β-actin was used as a loading control. B) Differential interference contrast micrographs of MDA-MB-231 cell morphology at different treatment. *Scale bars*, 10 μm. C) Cell death measured by CCK-8 assay at different treatment. Error bars indicated s.d. (n = 3). (***P<0.001). D) Concentration dependent cell death. Error bars indicated s.d. (n = 3).

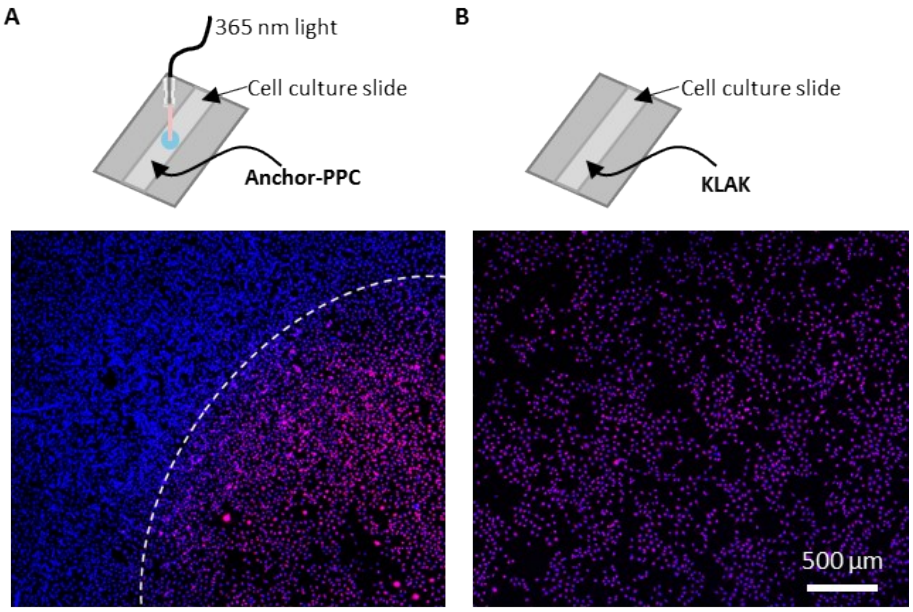


Figure S21. Spatial control of caspase-3 activation. **Anchor-PPC** (15 µM) are applied to a cell culture slide containing MDA-MB-231 cells. Fluorescence micrographs of the active caspase-3 (red) merged with Hoechest 33342-stained nuclei (blue) in A) **anchor-PPC** (15 µM) treated and B) KLAK treated MDA-MB-231 cells. Activation of caspase-3, seen in the form of an immunostained red fluorescence signal, was observed to occur exclusively in the light-irradiated area in **anchor-PPC** treated group. However, KLAK induced systematic activation of caspase-3. Each image is created by stitching 25 fluorescence images pictured by ×20 magnification confocal microscope.

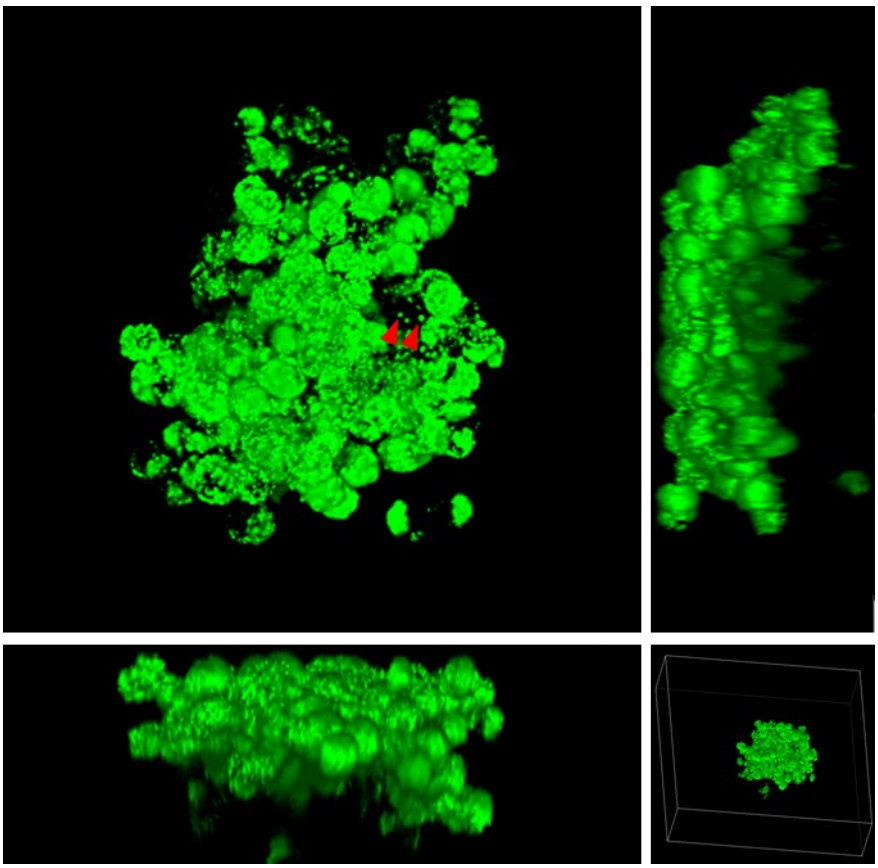


Figure S22. *In situ* molecular phase transition of **anchor-PPC** in 3D MDA-MB-231 spheroid.

References

[1] a) C. J. Ochs, G. K. Such, Y. Yan, M. P. van Koeverden, F. Caruso, *ACS Nano* **2010**, *4*, 1653-1663; b) B. Xu, C. Feng, X. Huang, *Nat. Commun.* **2017**, *8*, 333; c) J. Hu, K. Peng, J. Guo, D. Shan, G. B. Kim, Q. Li, E. Gerhard, L. Zhu, W. Tu, W. Lv, M. A. Hickner, J. Yang, *ACS Appl. Mater. Inter.* **2016**, *8*, 17499-17510.