Supporting Information

Self-Assembling Nanowires of an Amphiphilic Camptothecin Prodrug Deriving from Homologous Derivatives Conjugation

Experimental Section

Materials and methods

Bis(2-hydroxyethyl) disulfide were purchased from Beijing InnoChem Science & Technology Co., Ltd, China. Camptothecin and Irinotecan Hydrochloride were purchased from Dalian Mellon Biological Technology Co., Ltd, China. Triphosgene were purchased TCI (Shanghai) Development Co., Ltd, China. Dithiothreitol (DTT) was purchased from AMRESCO. 4-(dimethylamino) pyridine (DMAP) and all solvents, including anhydrous solvents, were obtained from J&K, China. Dichloromethane (DCM) was distilled over CaH2. Water was purified by a Milli-Q system (Millipore, Milford, MA, USA).

Fetal bovine serum (FBS), Dulbecco's modified eagle's medium (DMEM) and RPMI 1640 medium were purchased from Wisent Inc. (Quebec, Canada). LysoTracker was purchased from Thermo Fisher Scientific Inc. Also, 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich. All the cell lines used in this experiment were purchased from American Type Culture Collection (ATCC; Manassas, VA). Culture plates and dishes were bought from Corning (Corning, New York, USA). MCF-7 cells were cultured in DMEM with 10 % fetal bovine serum and 1 % antibiotic solution. A549 and HCT-8 cells were maintained in RPMI 1640, supplemented with 10 % fetal bovine serum and 1 % antibiotic. All the cell lines were cultured in a humidified atmosphere containing 5 % CO_2 at 37 °C.

The characteristics of CPT-ss-Ir were determined by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS, Microflex LRF, Bruker Dalton), nuclear magnetic resonance (NMR, AVANCE III, Bruker Biospin). UV absorbance spectra of the compounds were tested by a ultra-violet and visible spectrophotometer (Lambda 950, Perkin Elmer). Fluorescence spectra of the compounds were recorded by a fluorescence spectrophotometer (LS55, Perkin Elmer). Morphology and size of CPT-ss-Ir nanowires were observed under a transmission electron microscope (TEM, Hitachi HT7700). The surface charge of CPT-ss-Ir nanowires was measured by dynamic light scattering (DLS, Nano ZS90, Malvern, UK). The released CPT and Ir from CPT-ss-Ir was quantitated by high performance liquid chromatography (HPLC, Shimadzu C18 column 3 μm, 50 mm × 4.6 mm). MTT evaluation was monitored in a microplate reader (Infinite M200, Tecan). Cell confocal images were shot with a confocal laser scanning microscopy (CLSM, LSM710, Carl Zeiss).

Synthesis of intermediate CPT-ss-OH

Intermediate CPT-ss-OH was synthesized according to literature, In a 250 mL round

bottom flask, CPT (1.0 g, 2.87 mmol) and triphosgene (311.6 mg, 1.05 mmol) were suspended in anhydrous DCM (90 mL) under argon, DMAP (1.12 g, 9.18 mmol) dissolved in DCM (1 mL) was added drop wise to the system. After stirring at room temperature for 20 min, Bis (2-hydroxyethyl) disulfide (4.43 g, 28.7 mmol) in anhydrous THF (18 mL) was added. The solution was stirred at room temperature overnight. The mixture was washed with 0.1 M HCl (3 × 50 mL), brine (1 × 90 mL) and then with water (2 × 50 mL). The organic phase was dried with MgSO4. After the solvent was removed in vacuo, the residue was purified by three times re-crystallization from methanol/chloroform (10:3/v:v) to give a slight yellow solid (905 mg, yield 59.5 %).

Synthesis of CPT-ss-Ir

In a 25 mL round bottom flask, under argon, intermediate CPT-ss-OH (100 mg, 0.19 mmol) and triphosgene (20.8 mg, 0.07 mmol) were dispersed in anhydrous DCM (5 mL). DMAP (74.3 mg, 0.61 mmol) dissolved in DCM (0.4 mL) was added drop wise to the flask. The mixture was stirred at room temperature for 25 min. Ir (355.1 mg, 0.57 mmol) in anhydrous DCM (5 mL) was added and then the solution was stirred at room temperature for 24 h. After the solvent was removed under vacuum, the crude products was purified by silica gel column chromatography (EtOAc/MeOH = 25:1 to 5:1, v/v) to give a yellow solid (100 mg, yield 46.4 %). The molecular structure was determined by MALDI-TOF-MS and ^{1}H NMR.

Preparation of CPT-ss-Ir nanowires

We used three different methods to prepare CPT-ss-Ir nano-formulations. **Method 1**, nanoprecipitation method without ultra-sonication. In brief, 8 mg CPT-ss-Ir was dissolved in 1 mL dimethylsulfoxide (DMSO) and stirred for 2 min at room temperature. The solution was slowly injected to 4 mL deionized water under rigorous stir and then stirred slightly for 15 min. Subsequently, the mixture was dialyzed (MWCO = 500 Da) against deionized water for 24 h to remove DMSO. Finally, CPT-ss-Ir nanowires with a concentration of 877 μM were prepared. **Method 2**, nanoprecipitation method with ultra-sonication. In brief, 5.0 mg of CPT-ss-Ir was dissolved in 50 μL dimethylsulfoxide (DMSO) and then the mixture was added drop wise to 4.95 mL deionized water under ultrasound to reprecipitate CPT-ss-Ir. Finally, a stable nano-formulated CPT-ss-Ir was obtained with a final concentration of 877 μM. **Method 3**, emission method. The emusion was formed between an organic solution (5 mg CPT-ss-Ir in 500 μL methylene chloride) and an aqueous solution (5 ml of an aqueous solution) using sonication, and then the organic solution was removed quickly using a rotating evaporator. Finally, stable CPT-ss-Ir nanowires were obtained with a concentration of 877 μM. The TEM samples were prepared by diluting the solution to 50 μM and dropping onto copper grids. After drying in the air, the TEM observation was carried out under a transmission electron microscope (TEM, Hitachi HT7700) at operation voltage of 120 kV.

Degradation study of nanowires

Three different methods were used to confirm the degradation of nanowires.

TEM test: Before DTT treatment, the morphology and size of CPT-ss-Ir nanowires were

observed by TEM. Then DTT was added with a final concentration of 10 mM. The morphology was evaluated by TEM after incubating at 37 °C for 4 h and 12 h, respectively. Fluorescence (FL) intensity test: FL intensity changes of CPT-ss-Ir (50 μM) upon DTT treatment (10 mM) for up to 4 h were measured.
Thin-layer chromatography (TLC) test: CPT-ss-Ir nanowires were treated with DTT for 10 mins, and EtOAc/MeOH (10:1/v:v) were used as developing solvent. The TLC result was shown under UV light.

In vitro CPT release from CPT-ss-Ir nanowires

The CPT-ss-Ir nanowires were diluted with water to 50 μM. Then 10 mM DTT was added and the solution was incubated at 37 °C. At indicated time points, 1.0 mL solution was collected and brought to HPLC analysis, Gradient method (0.1 % TFA-H_2O/Acetonitrile, see Fig. S6) was adopted to elute CPT, Ir and CPT-ss-Ir in single run.

Cell culture

MCF-7 cells were cultured in Dulbecco's Modified Eagle's medium (DMEM), A549 and HCT-8 cells were cultured in RPMI 1640 medium at 37 °C under a humidified atmosphere containing 5 % CO2. The culture mediums contain 10 % fetal bovine serum (FBS) and 1 % antibiotics.

Cellular Uptake and Intracellular Drug Release

MCF-7 cells were seeded in 35 mm dish with a glass bottom at 2.0 $\times 10^5$ cells in 3 mL of complete DMEM and incubated for 24 h, followed by treating with drug solutions at the concentration of 5 μM for 5 h at 37 °C. Cells were then washed with PBS for two times and imaged using a confocal laser scanning microscopy (LSM710, Carl Zeiss) with excitation at 405 nm for all these drugs. For intracellular drug release monitoring, MCF-7 cells were first incubated with CPT-ss-Ir nanowires (50 μM) for just 0.5 h and replaced with fresh medium. Then CLSM images were taken at different time points after further incubation.

Cell viability assay

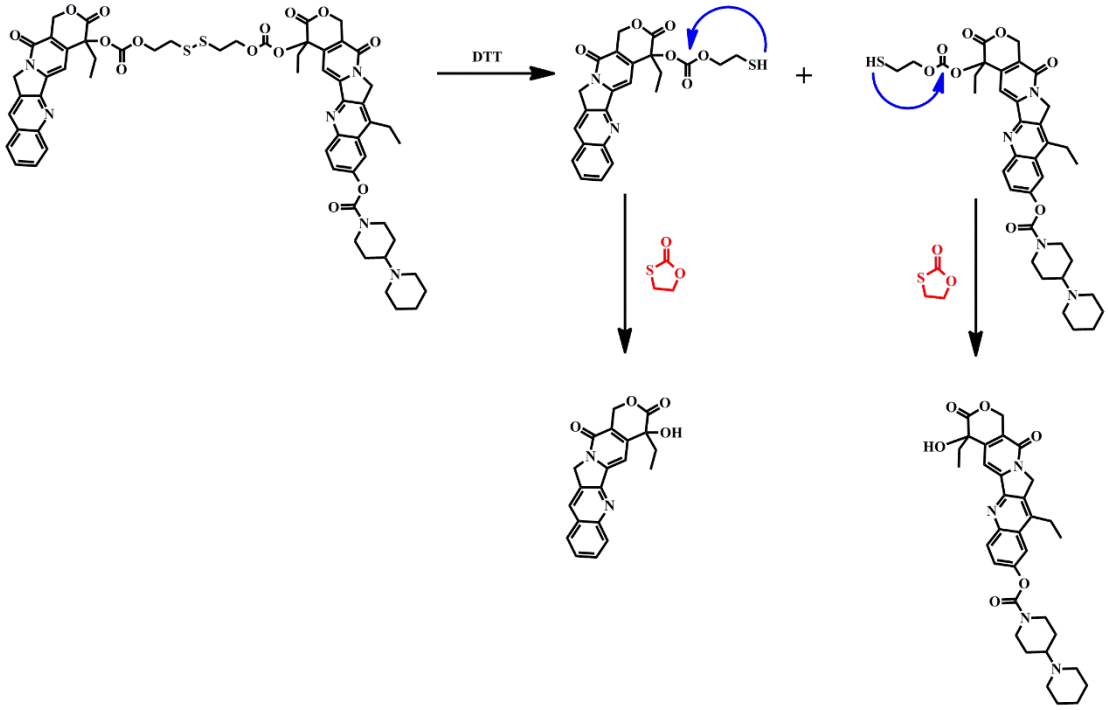
The cytotoxicity of CPT-ss-Ir nanowires was measured by MTT assay using a microplate reader (Tecan infinite M200). Cells were seeded in a 96-well plate at a density of 5×10^3 cells per well and pre-incubated for 24 h, then incubated with free CPT, free Ir and CPT-ss-Ir nanowires for 48 h at various concentrations ranging from 0.1 to 10 μM. After that the medium was replaced by 100 μL fresh culture medium containing MTT (0.5 mg/mL) and cells were incubated for another 4 h. Afterward, the MTT medium was replaced by 150 μL of DMSO to dissolve the formazan crystals formed by living cells. Finally, the plates were analyzed using a microplate reader with the absorbance at 570 nm and with 630 nm as the reference wavelength. Cells without drugs treatment were used as control and each concentration was performed with 5 duplicate.

References

1. J. Liu, W. Liu, I. Weitzhandler, J. Bhattacharyya, X. Li, J. Wang, Y. Qi, S. Bhattacharjee

and A.Chilkoti, *Angewandte Chemie International Edition*, **2015**, 54, 1002-1006.

Supporting Figures



Scheme S1 The schematic illustration of the drug release process: the cleavage of the disulfide bond and cyclization reactions under the reductant.

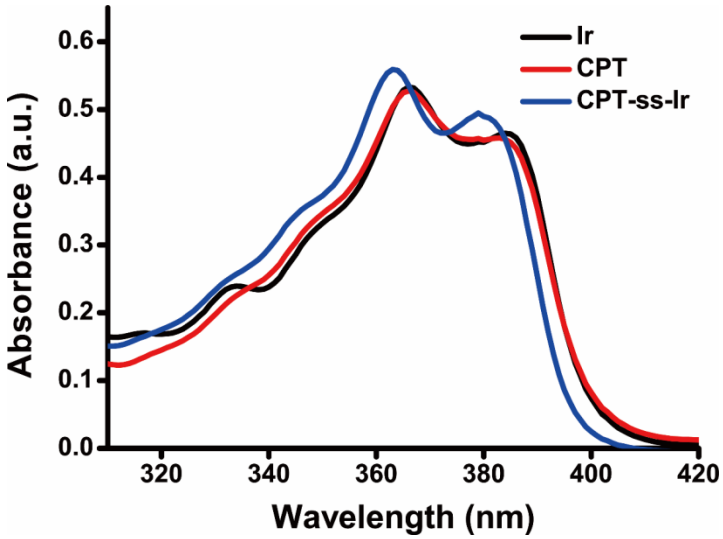


Fig. S1 UV-vis spectra of Ir, CPT and CPT-ss-Ir in DMSO.

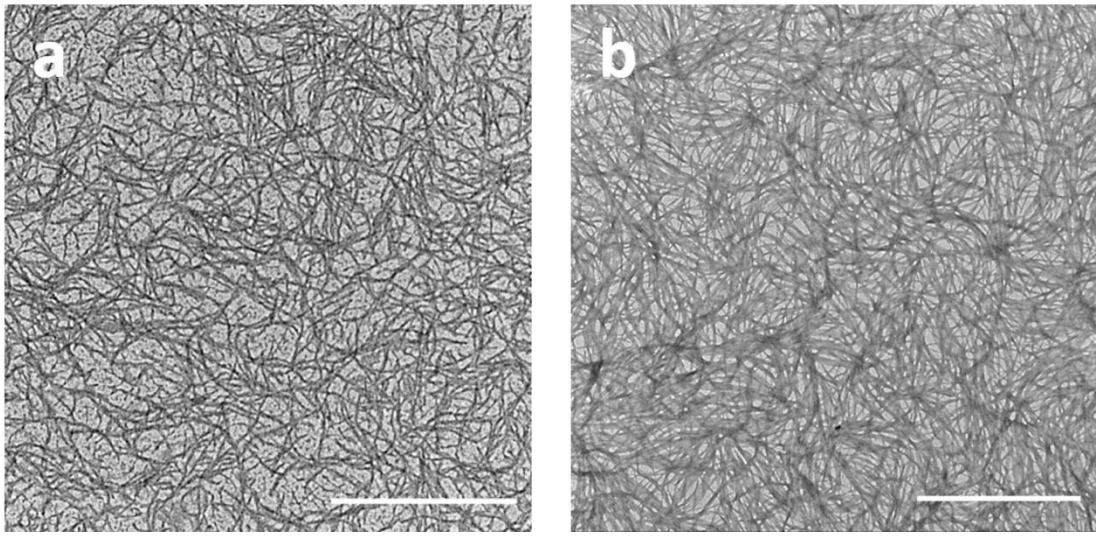


Fig. S2 TEM images of CPT-ss-Ir nanowires prepared by method 2 and method 3. Scale bar, 1 μm.

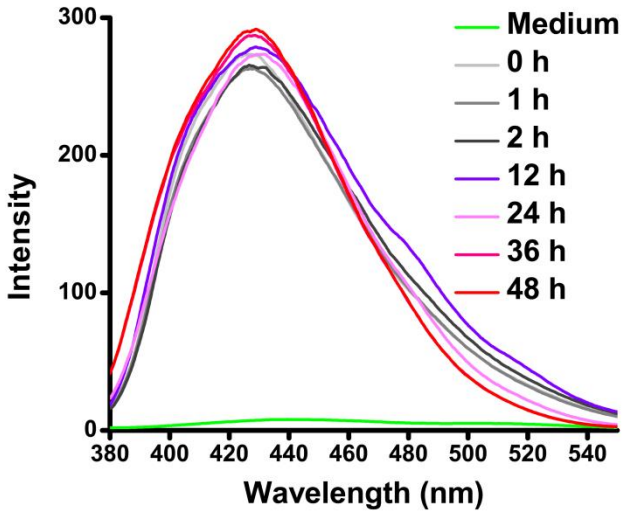


Fig. S3 Fluorescence spectra of CPT-ss-Ir nanowires in culture medium containing FBS at different time points.

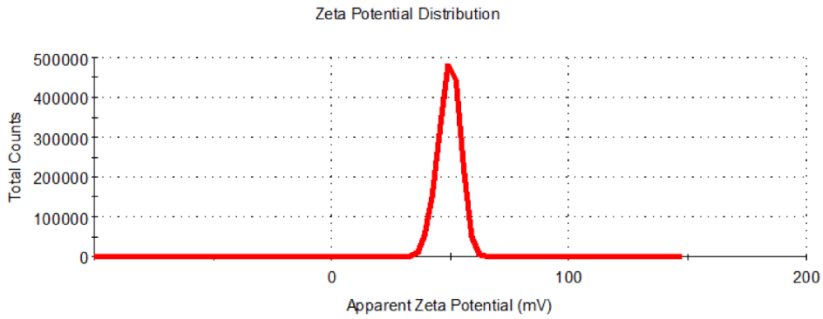


Fig. S4 The zeta potential of CPT-ss-Ir nanowires in water.

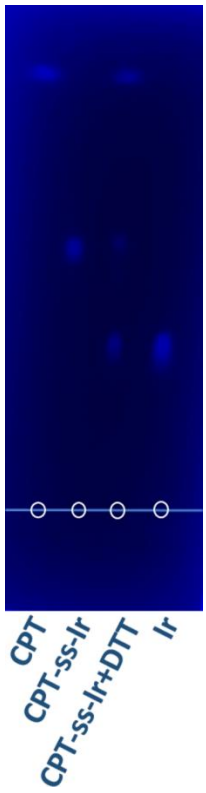


Fig. S5 Image of TLC result to confirm the release of CPT and Ir after treated by DTT.

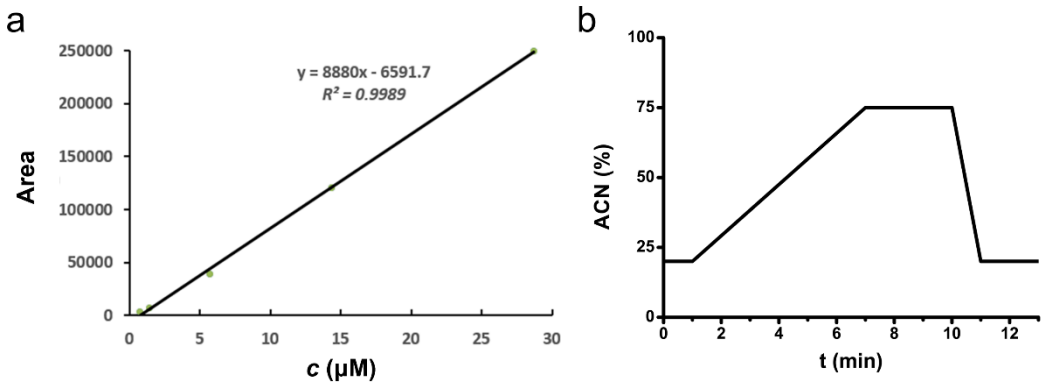


Fig. S6 HPLC standard curve of CPT (a); Time line of HPLC method as shown by acetonitrile gradient change (b).

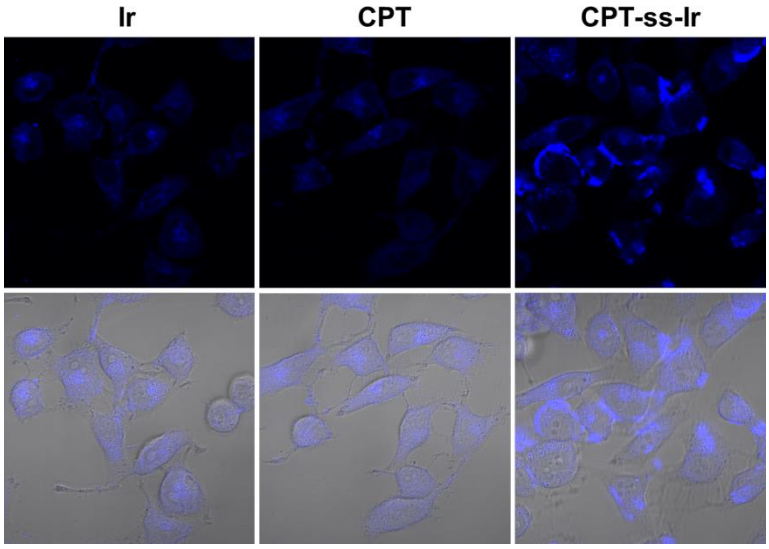


Fig. S7 Confocal microscopy images of MCF-7 cells after incubated with CPT, Ir or CPT-ss-Ir.

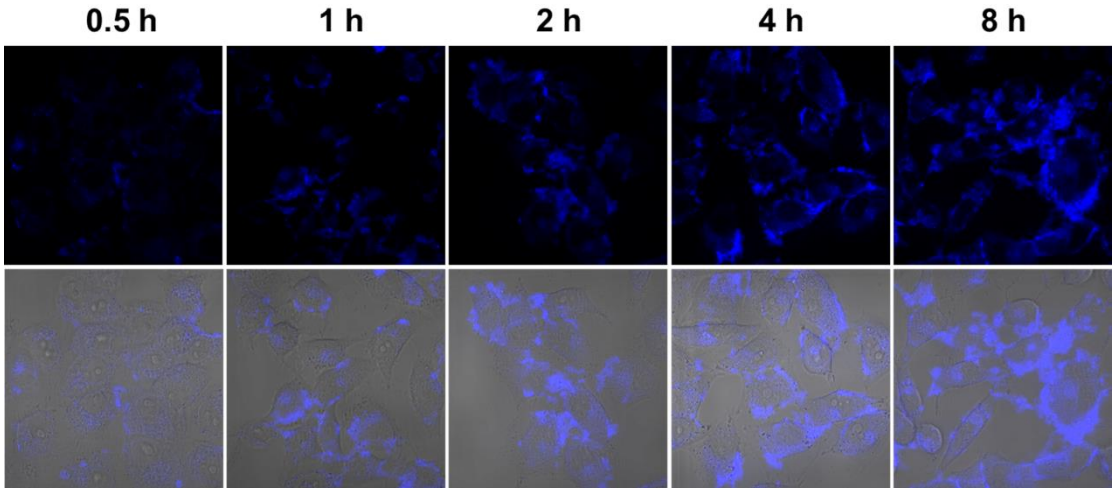


Fig. S8 Confocal microscopy images of MCF-7 cells after treated with CPT-ss-Ir nanowires for 30 min and then incubating further for different time points.