Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Supramolecular Assembly Mediated by Host-Guest Interaction for Improved Chemo-Photodynamic Combination Therapy

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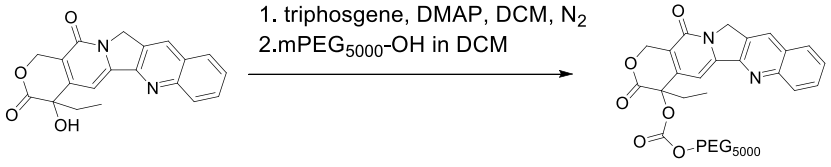
General experimental

All reagents and solvents were purchased from commercial suppliers and were used without further purification. NMR spectra were recorded on an AVANCE III operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Mass spectrometry was performed using a Bruker MicrOTOF instrument (Electro-Spray Ionization). Fluorescence microscopy was carried out on a DMi 8 microscope (Leica, Germany). UV-Vis absorbance spectroscopy was measured on an Agilent Cary 100 UV. Fluorescence spectroscopy was measured on Shimadzu RF-6000. Dynamic light scattering (DLS) and ζ-potential measurements were carried on a Malvern ZetaSizer Nano-ZS. Transmission electron microscopy (TEM) measurements were performed on a Tecnai G2 F20 S-Twin apparatus operating at an accelerating voltage of 200 kV. Confocal laser scanning microscopy (CLSM) images were performed on a Nikon C2þ laser scanning confocal microscope (Nikon, Japan).

Synthesis and Characterizations

TPP-OH **CPT-SS-OH** **CPT-SS-TPP**

CPT-SS-TPP: TPP-OH and CPT-SS-OH were synthesized according to literature reports.[1,2] CPT-SS-OH (100 mg, 0.19 mmol) and triphosgene (20.8 mg, 0.07 mmol) were dissolved in CH_2Cl_2 (5 mL) under N_2 protection at room temperature. Dimethylaminopyridine (74.3 mg, 0.61 mmol) in CH_2Cl_2 (1 mL) was added into the solution and stirred for 0.5 h. TPP-OH (362 nm, 0.57 mmol) in CH_2Cl_2 (5 mL) was slowly added into the solution and stirred overnight. The solution was evaporated and the residue was purified by silica gel column chromatography (CH_2Cl_2 and CH_3OH) to afford CPT-SS-TPP (112.2 mg, 0.9 mmol, 49.8%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.87-8.79 (m, 8H), 8.28 (s, 1H), 8.28-8.21 (m, 8H), 8.06 (d, *J* = 7.6 Hz, 1H), 7.82-7.75 (m, 11H), 7.58 (d, *J* = 8.0 Hz, 1H), 7.55 (d, *J* = 7.6 Hz, 2H), 7.38 (s, 1H), 5.73 (d, *J* = 16.8 Hz, 1H), 5.40 (d, *J* = 16.8 Hz, 1H), 5.26 (s, 2H), 4.59 (t, *J* = 6.0 Hz, 2H), 4.46 (t, *J* = 6.0 Hz, 1H), 3.12 (t, *J* = 6.4 Hz, 2H), 3.06 (t, *J* = 6.4 Hz, 2H), 2.33-2.30 (m, 1H), 2.21-2.16 (m, 1H), 1.02 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ = 167.5, 156.9, 153.3, 152.6, 151.1, 148.2, 146.6, 145.2, 141.6, 139.4, 136.0, 135.5, 134.6, 132.5, 132.4, 132.2, 131.9, 130.8, 130.0, 129.4, 129.3, 128.8, 128.5, 128.3, 128.0, 127.4, 120.6, 120.5, 120.1, 119.6, 119.2, 114.4, 94.8, 78.4, 66.9, 66.8, 66.7, 50.6, 36.8, 36.7, 30.8, 8.0. HR-MS: m/z 1185.3296 ([M+H$^+$], calculated for $[C_{70}H_{53}N_6O_9S_2]^+$, 1185.3315).



CPT **CPT-mPEG**

CPT-mPEG: CPT (200 mg, 0.57 mmol) and triphosgene (63 mg, 0.21 mmol) were dissolved in CH_2Cl_2 (20 mL) under N_2 protection at room temperature. Dimethylaminopyridine (202 mg, 1.65 mmol) in CH_2Cl_2 (2 mL) was added into solution and stirred for 1 h. mPEG-OH (1.4 g, molecular weight = 5000) were dissolved in CH_2Cl_2 (5 mL) and added into the reaction mixture. After 2 days stirring, CH_2Cl_2 was removed by evaporation. Water (40 mL) was added into the mixture and centrifuge for 15 min (12000 rpm). The supernatant was collected and dialysis (MWCO = 5000) against water for 2 days and followed by freezing dry to afford CPT-mPEG (1.1 g, 72.8%). ^{1}H NMR (D_2O, 400 MHz): δ = 8.11 (s, 1H), 7.77 (d, *J* = 8.8 Hz, 1H), 7.63-7.57 (m, 2H), 7.37 (t, *J* = 7.6 Hz, 1H), 7.21 (d, 1H), 7.54 (d, *J* = 16.8 Hz, 1H), 5.41 (d, *J* = 16.8 Hz, 1H), 4.33 (s, 2H), 3.78-3.41 (m, 447H), 3.27(s, 3H), 2.22-2.11 (m, 2H), 0.99 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (D_2O, 100 MHz): δ = 169.5, 157.0, 153.8, 150.1, 147.0, 146.5, 145.1, 132.1, 131.0, 127.9, 127.7, 127.4,

127.3, 118.8, 114.4, 97.0, 78.6, 71.7, 71.0, 69.4, 68.4, 68.2, 67.3, 60.3, 58.0, 30.6, 7.3.

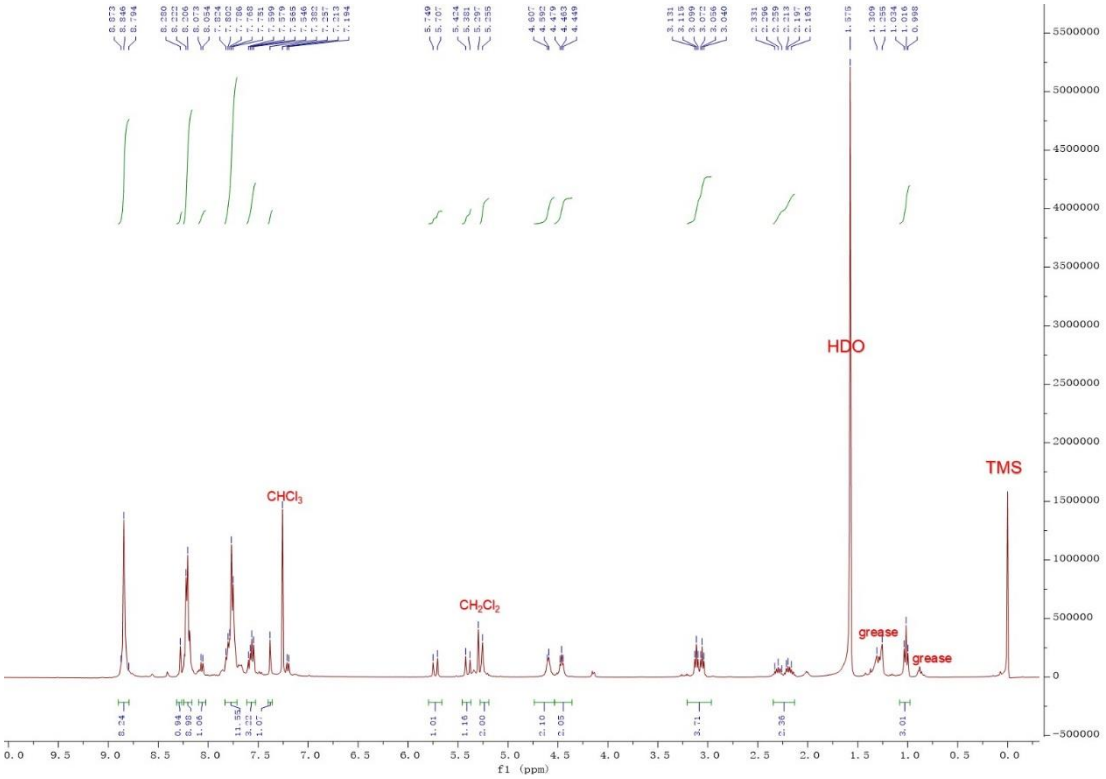


Figure S1. ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) recorded for CPT-SS-TPP.

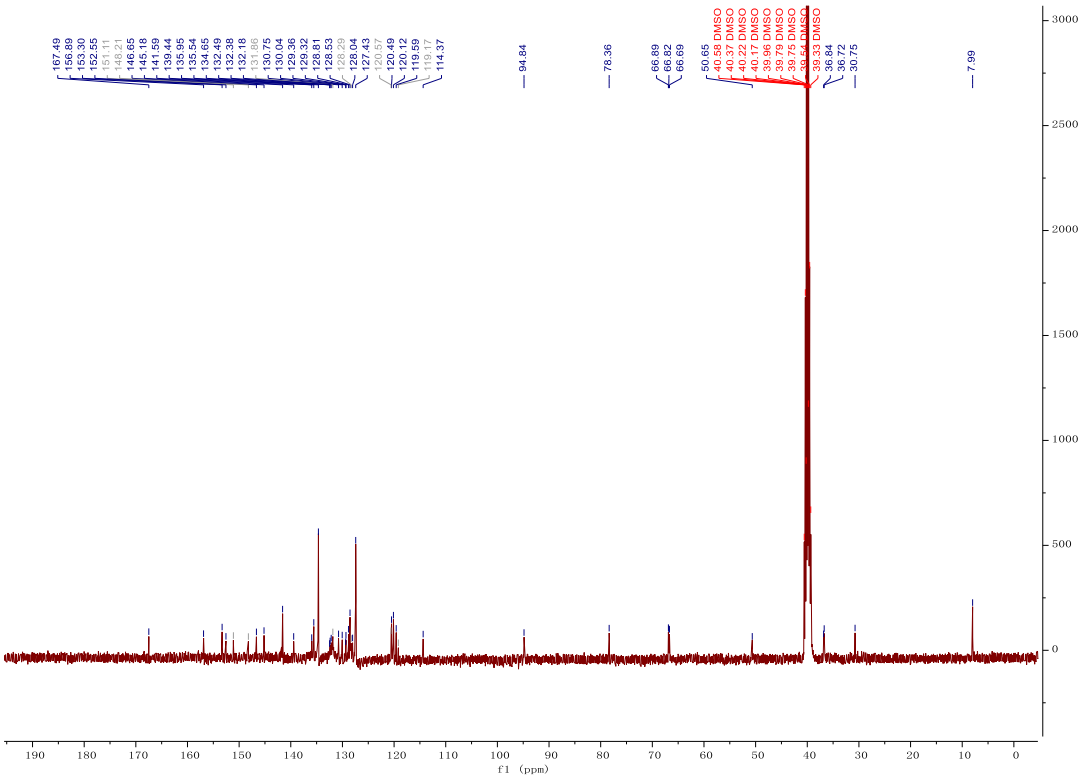


Figure S2. ^{13}C NMR spectrum (DMSO-d_6, 100 MHz) recorded for CPT-SS-TPP.

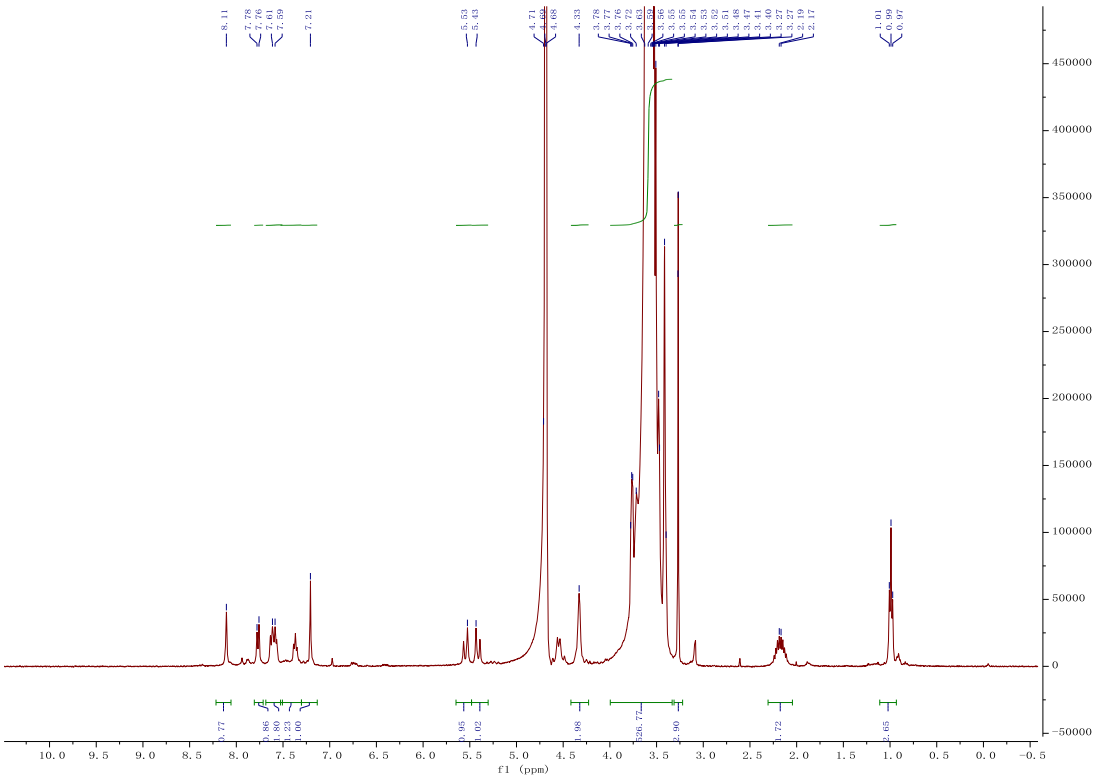


Figure S3. ^{1}H NMR spectrum (D_2O, 400 MHz) recorded for CPT-mPEG.

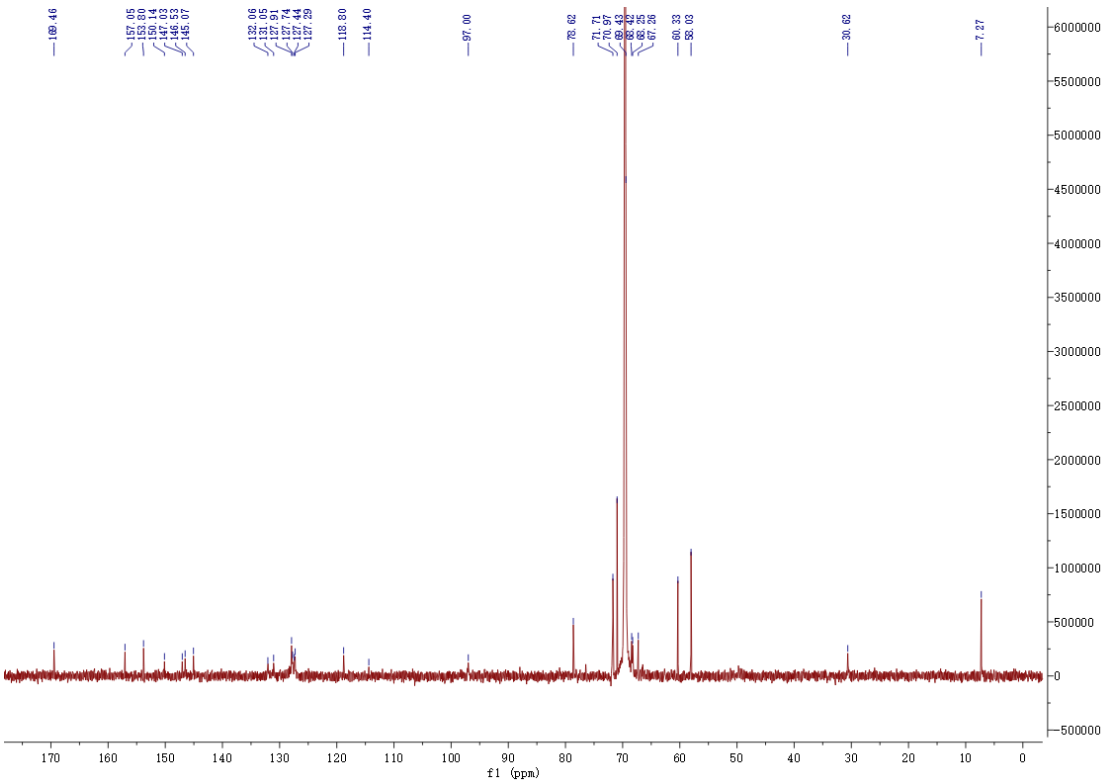


Figure S4. ^{13}C NMR spectrum (D_2O, 100 MHz) recorded for CPT-mPEG.

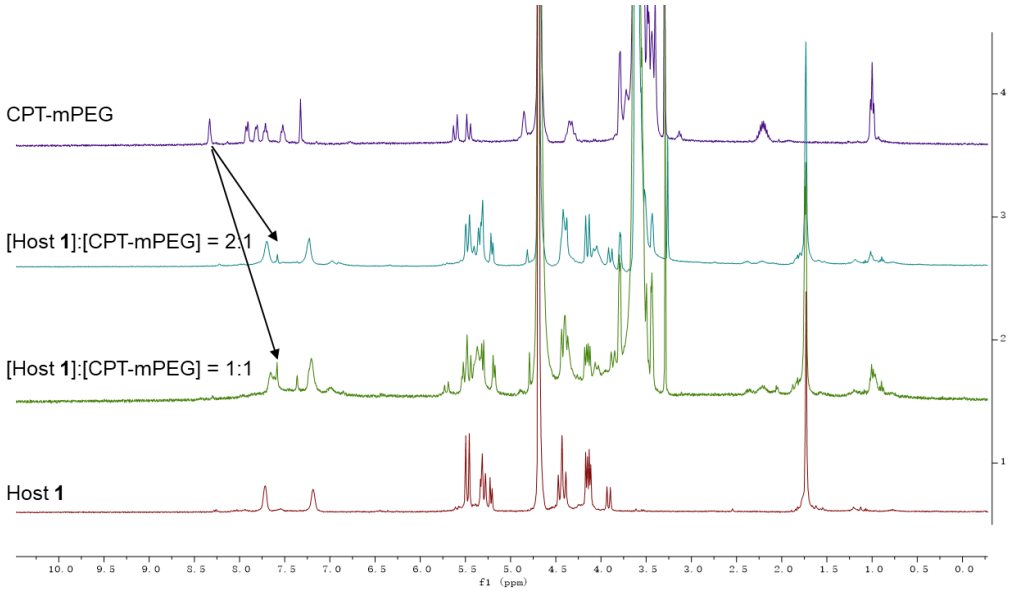


Figure S5. ^{1}H NMR spectrum (D_2O, 400 MHz) recorded for host **1**, CPT-mPEG and **1**•CPT-mPEG complex with different molar ratios.

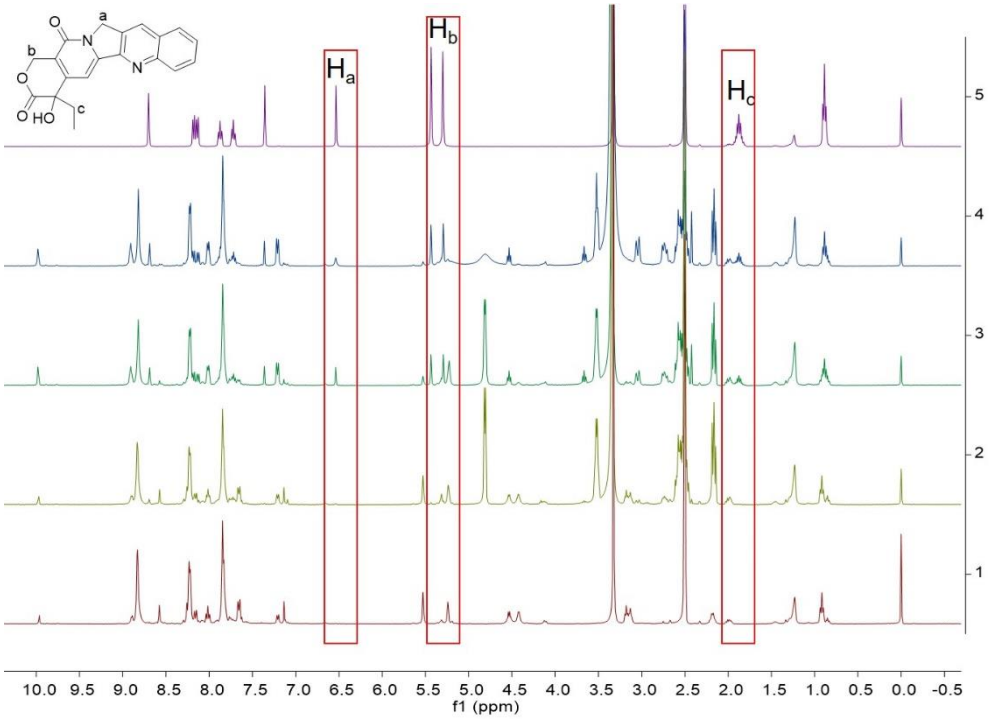


Figure S6. ^{1}H NMR spectrum (DMSO-d_6,400 MHz) recorded for (1) CPT-SS-TPP, DTT (10 mM) was added into the solution of CPT-SS-TPP for (2) 5min, (3) 5 h, (4) 24 h and (5) CPT. Red rectangle highlight the transformation of CPT-SS-TPP and the formation of CPT under 10 mM DTT.

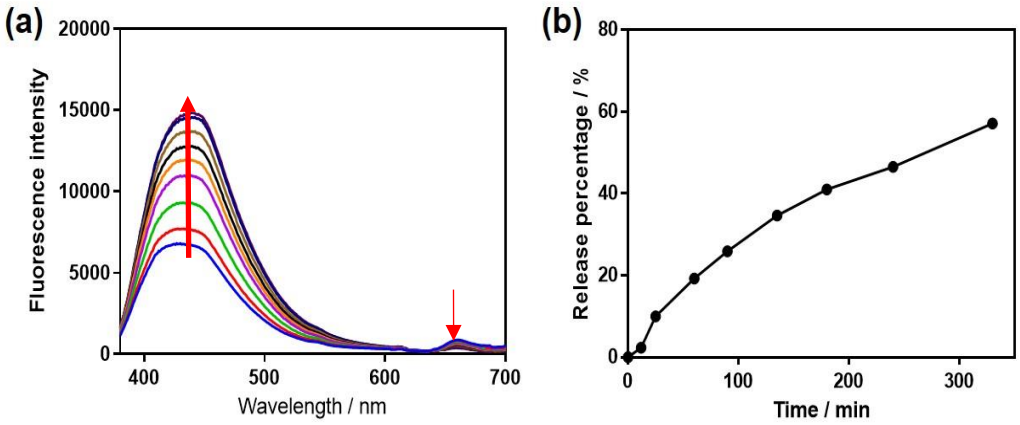


Figure S7. (a) Fluorescence spectra recorded for CPT-SS-TPP (1 μM in water, ex: 360 nm) in the presence of DTT (10 mM) at different time intervals (0 – 330 min), FRET peak is observed at 660 nm. (b) Release curve calculated based on disulfide cleavage rate (FRET peak at 660 nm).

Host-guest chemistry

Binding constants between guest and host **1** were calculated with nonlinear fitting with Origin 9 software based on 1:1 Binding and the following equation:
$[H]^0$: initial host concentration; $[G]^0$: initial guest concentration; Δ_A: change of absorbance or fluorescence intensity; Δ_ε: change of coefficiency; K_a: binding constant.

$$\Delta_A = \Delta_\varepsilon \cdot \{\frac{1}{2}([H]^0 + [G]^0 + \frac{1}{K_a}) - \sqrt{\frac{1}{4}([H]^0 + [G]^0 + \frac{1}{K_a})^2 - [H]^0 \cdot [G]^0}\}$$

K_a was evaluated by nonlinear fitting.

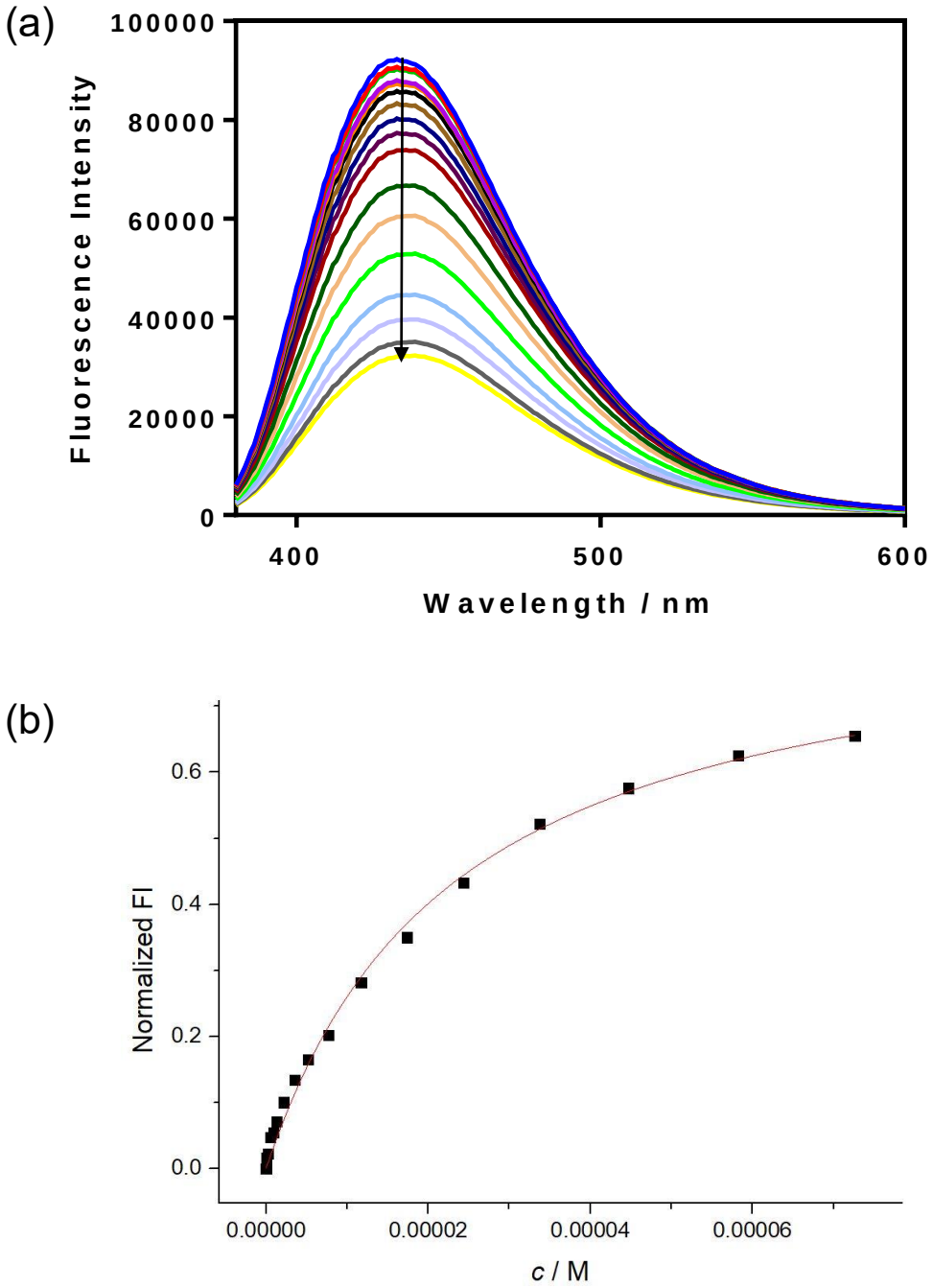


Figure S8. (a) Fluorescence emission spectra from the titration of CPT (1 μM) with host **1** (0 – 72.6 μM) in PBS (pH 7.4); (b) plot of ΔI at 430 nm as a function of the concentration of host **1**. Solid line represents the best non-linear fitting of the data based on a 1:1 binding model (K_a = (4.5 ± 0.3) × 10^4 M^{-1}).

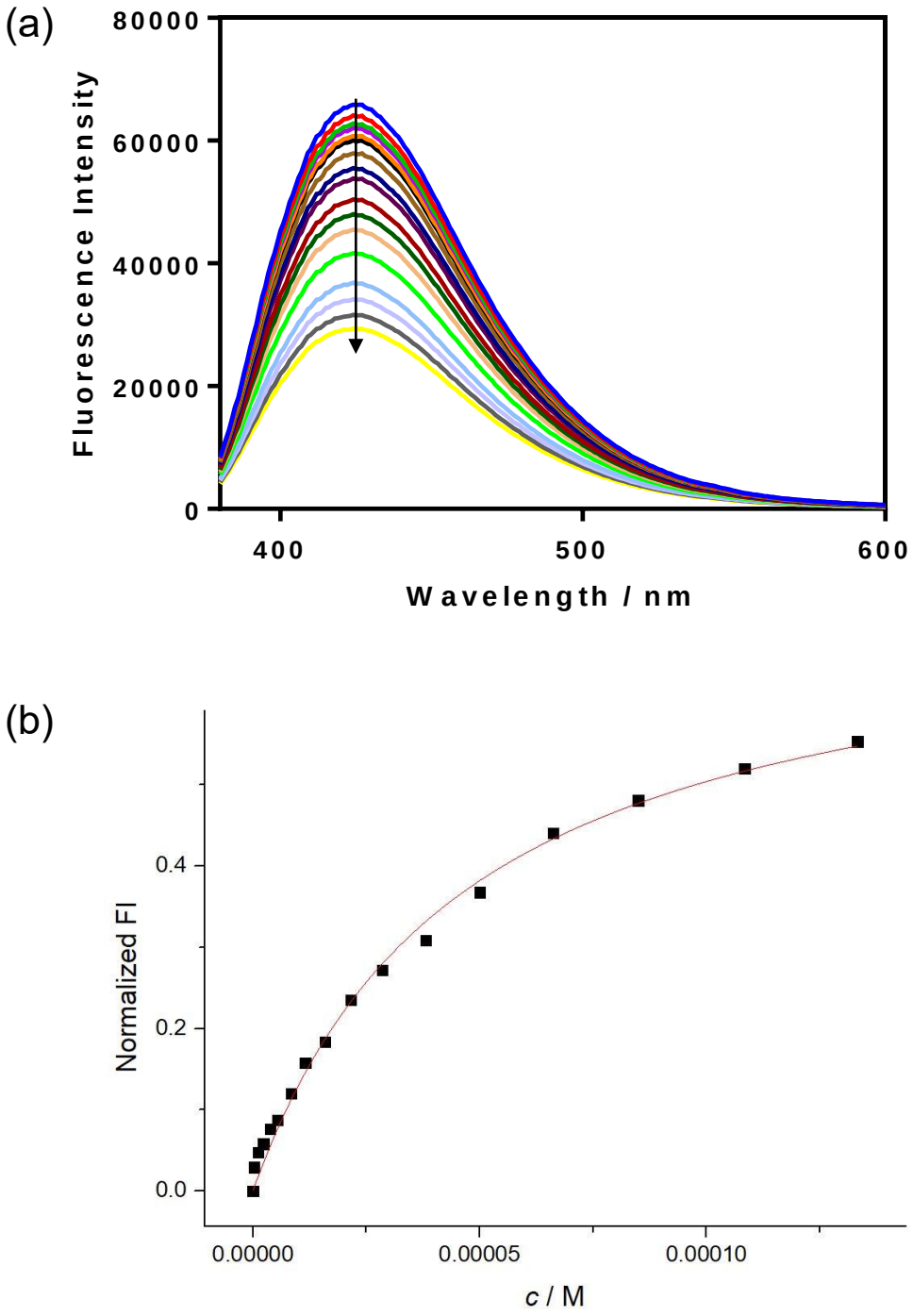


Figure S9. (a) Fluorescence emission spectra from the titration of CPT-mPEG (1 μM) with host **1** (0 – 133.2 μM) in PBS (pH 7.4); (b) plot of ΔI at 420 nm as a function of the concentration of host **1**. Solid line represents the best non-linear fitting of the data based on a 1:1 binding model (K_a = (2.2 ± 0.2) × 10^4 M^{-1}).

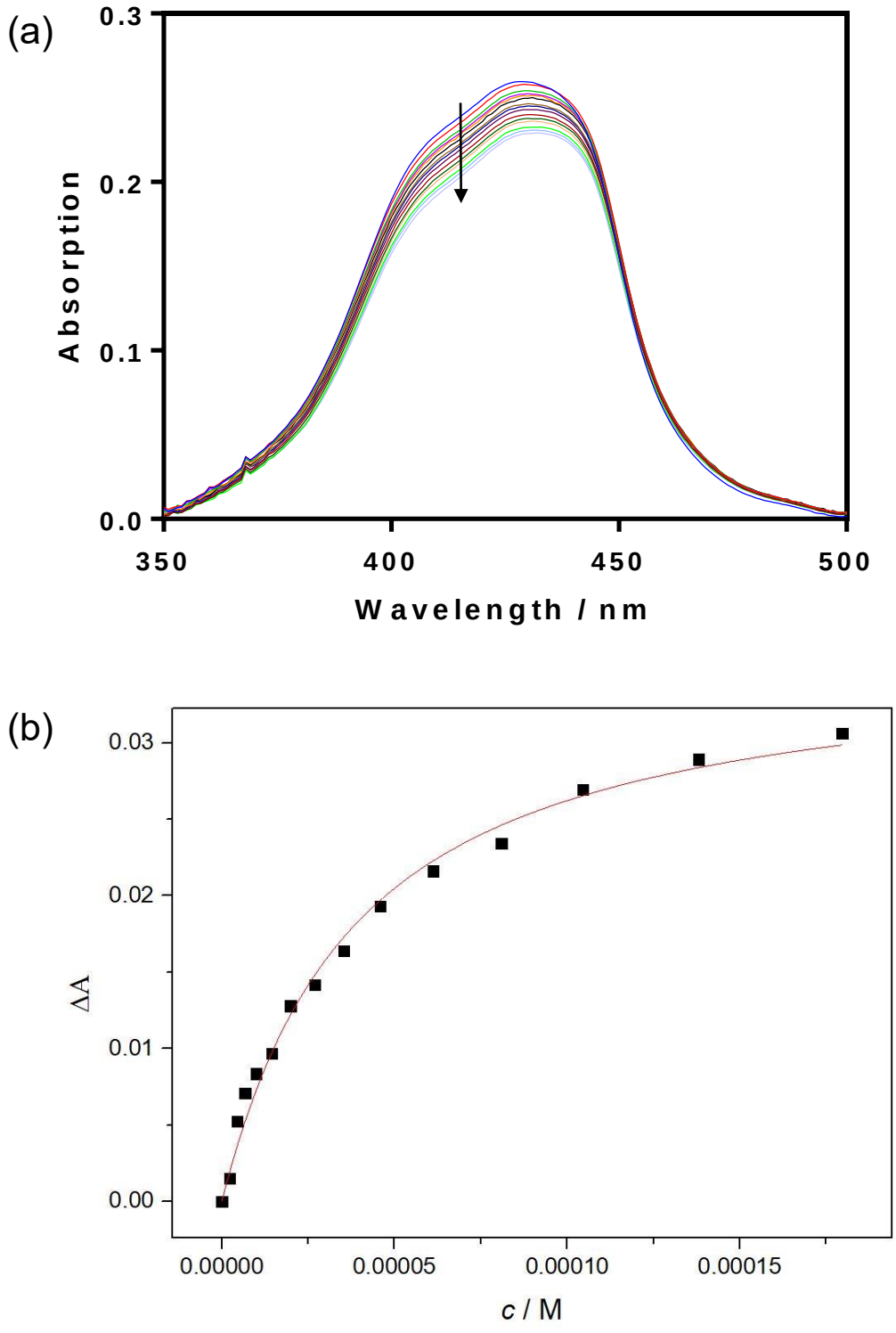


Figure S10. (a) Absorbance spectra from the titration of TPP-OH (20 μM) with host **1** (0 – 180 μM) in PBS (pH 7.4); (b) plot of ΔA at 430 nm as a function of the concentration of host **1**. Solid line represents the best non-linear fitting of the data based on a 1:1 binding model (K_a = (2.7 ± 0.2) × 10^4 M^{-1}).

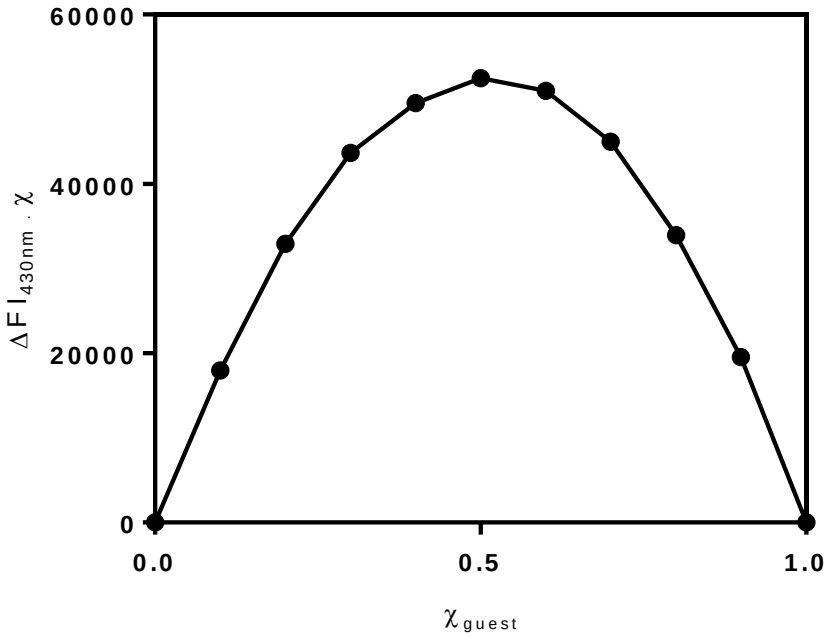


Figure S11. Job plot for the **1**·CPT complex in water. [host **1**] + [CPT] = 2 μM.

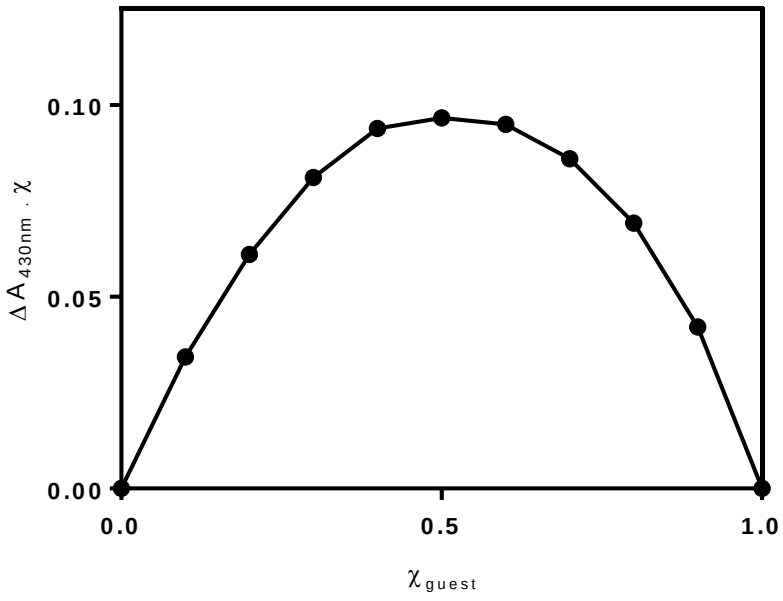


Figure S12. Job plot for the **1**·TPP-OH complex in water. [host **1**] + [TPP-OH] = 20 μM.

Self-assembly behavior

Preparation of SNM-1 and SNM-3. Due to the limited solubility of CPT-SS-TPP in water, a solution of CPT-SS-TPP (2 mM in DMSO) was injected into ultrapure water slowly under vigorous stirring in the presence or absence of host **1** and CPT-mPEG to yield SNM-1 and SNM-3 respectively. The final concentration of CPT-SS-TPP was 100 μM. The solution was dialyzed (MWCO = 14000) against water to remove DMSO, and used for further characterizations and experiments.

Determination of Critical Aggregation Concentration of self-assembly. In a typical experiment, SNM-3 (100 μM) stock solution was prepared. SNM-3 solution was diluted, and recorded by fluorescence spectroscopy. The fluorescence intensity of FRET peak at 660 nm was plot against molar concertation. The saltation was regarded as CAC.

Detection of $^1O_2$ generation in aqueous solution.

In a typical experiment, SNM-3 (10 μM) was suspended in water or FBS (10% in PBS) containing 30 μM DPBF. The solution was irradiated at 671 nm (0.2 W/cm^2). The UV-Vis spectra of the solution was recorded at different irradiation time intervals.

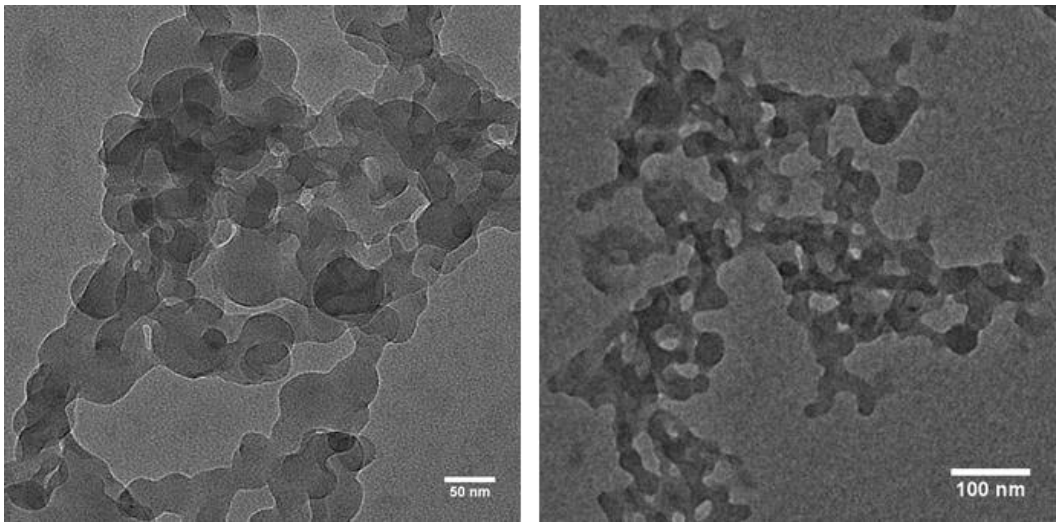


Figure S13. TEM images of SNM-1.

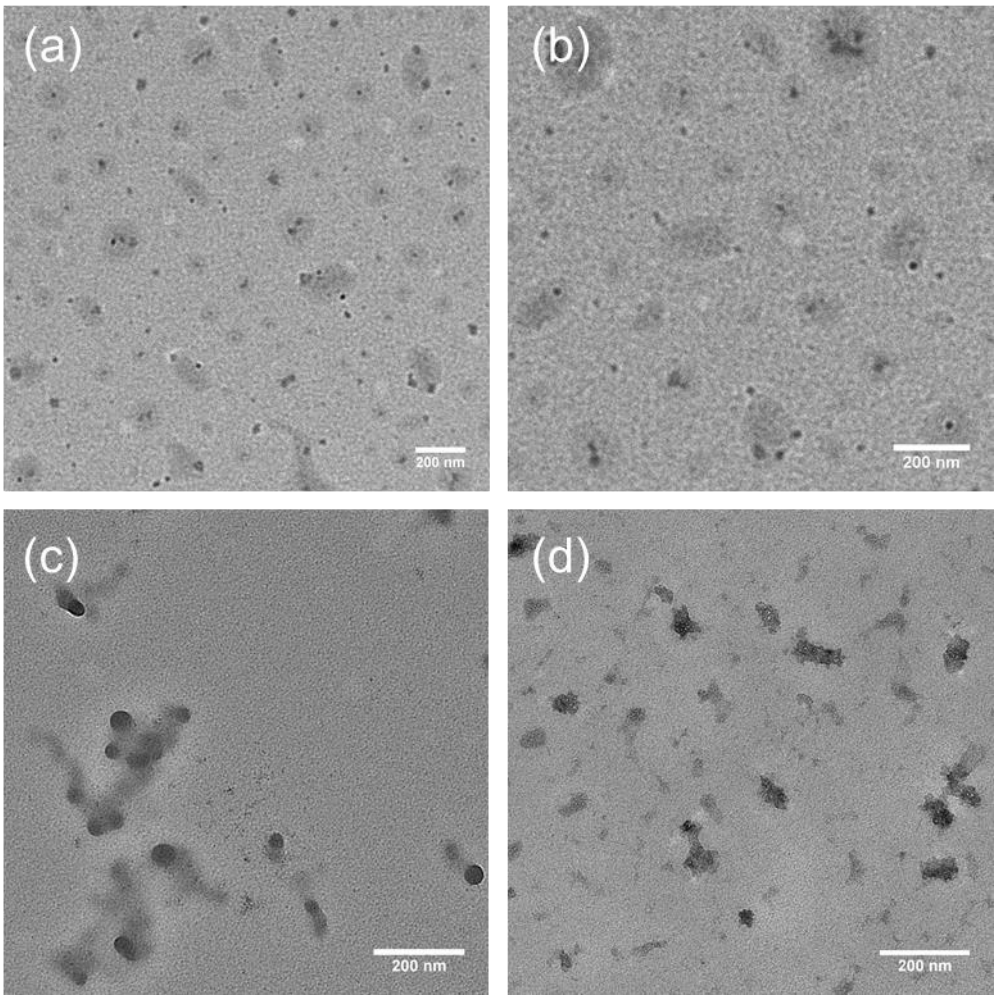


Figure S14. TEM images of CPT-SS-TPP in the presence of CPT-mPEG. (a), (b) [CPT-SS-TPP]:[CPT-mPEG] = 1:1. (c), (d) [CPT-SS-TPP]:[CPT-mPEG] = 10:1.

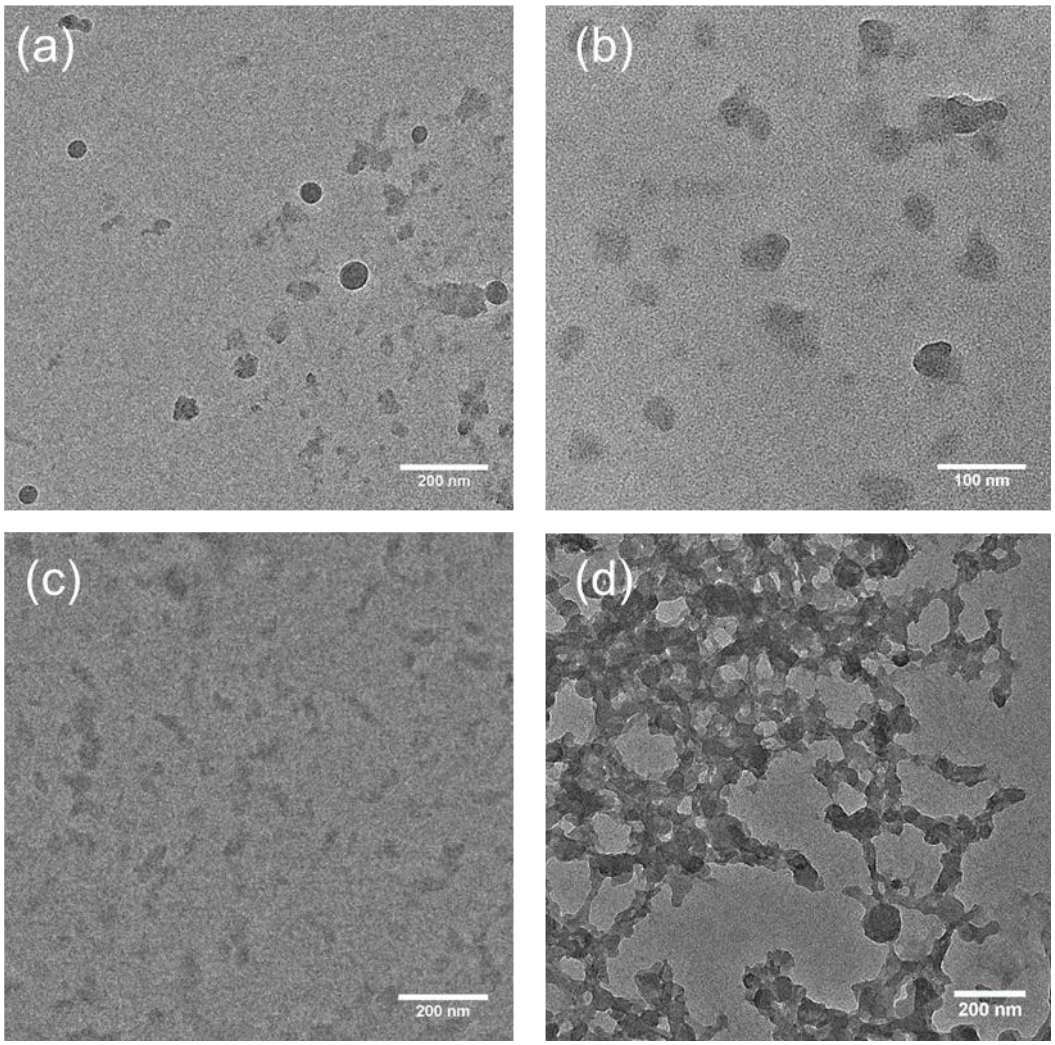


Figure S15. TEM images of CPT-SS-TPP in the presence of host **1** and CPT-mPEG, [CPT-SS-TPP]:[CPT-mPEG]:[host **1**]: (a) 0.5:2:2.5; (b) 1.25:0.5:1.75; (c) 1:1:5; (d) 1:0:1.

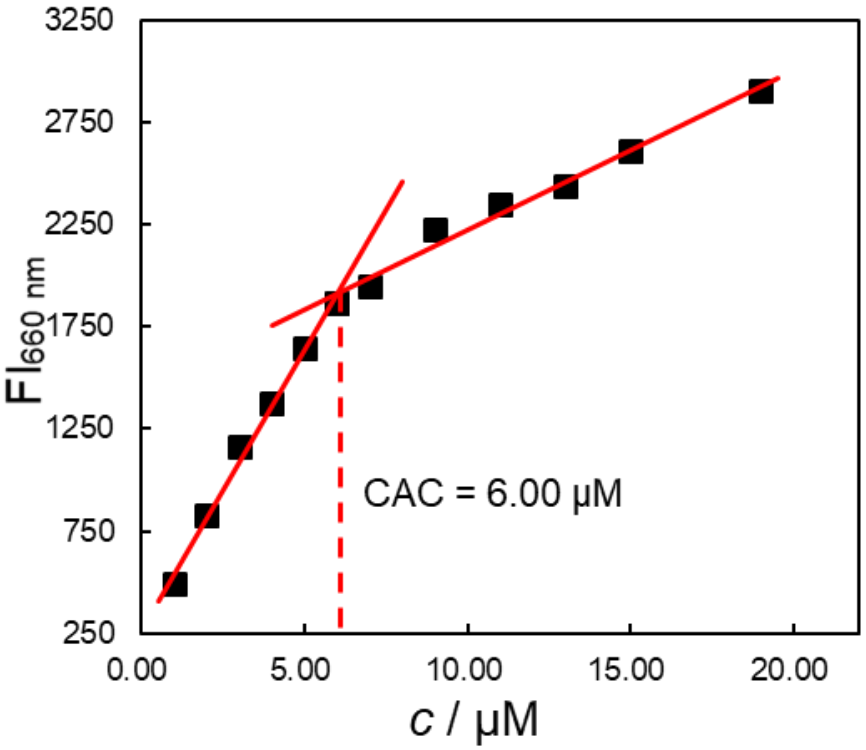


Figure S16. Fluorescence intensity against concentration recorded for SNM-1 in water (ex: 360 nm, em: 660 nm).

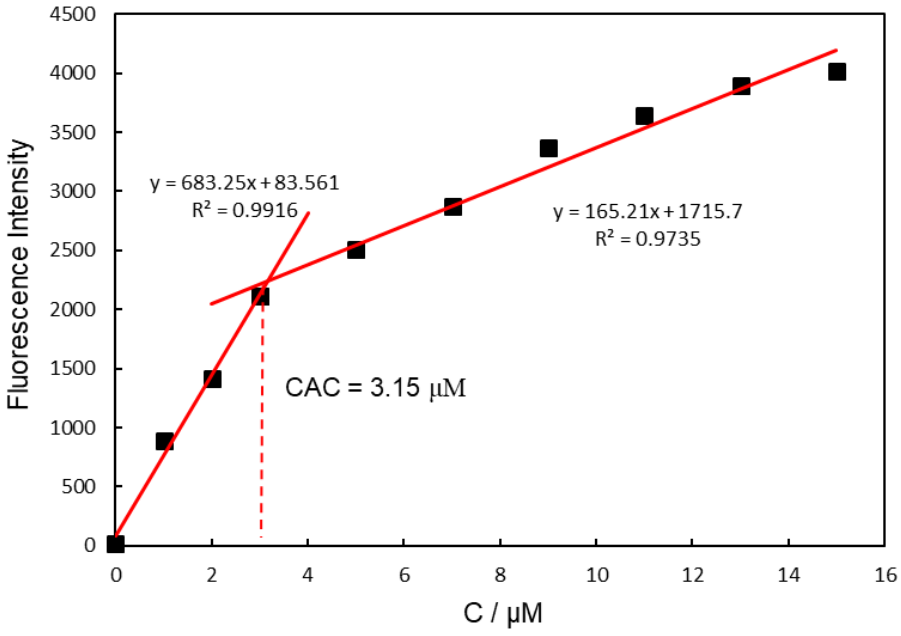


Figure S17. Fluorescence intensity against concentration recorded for SNM-3 in water (ex: 360 nm, em: 660 nm).

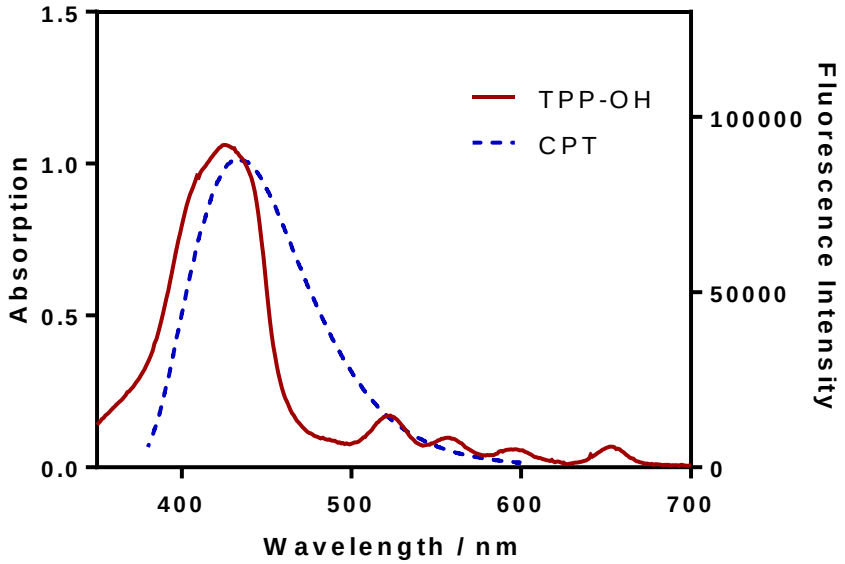


Figure S18. UV-Vis absorbance spectra of TPP-OH (red solid line) and fluorescence emission spectra of CPT (blue dash line, ex: 360 nm).

Stability evaluation

SNM-1 (10 μM) or SNM-3 (10 μM) were incubated in water or FBS (10%) at 37 ℃.

The fluorescence spectra and hydrodynamic size were recorded by fluorescence spectroscopy and DLS at different time intervals.

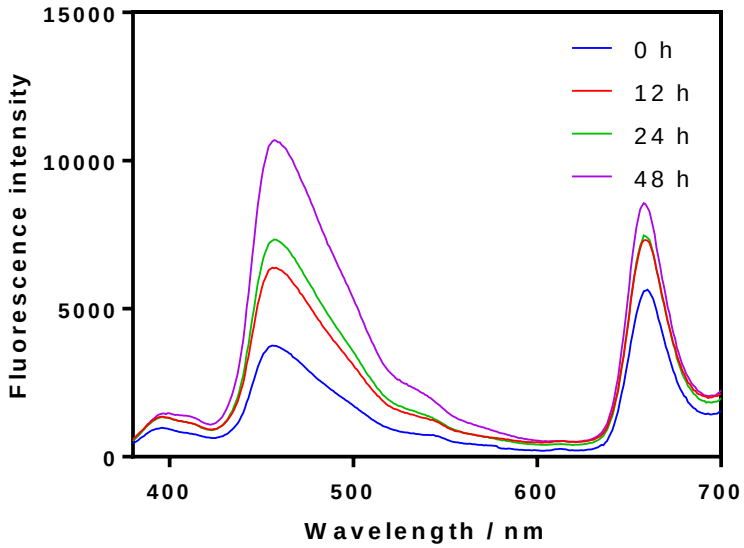


Figure S19. Fluorescence emission spectra recorded for SNM-1 (10 μM) in 10% FBS at different incubation time intervals (ex: 360 nm).

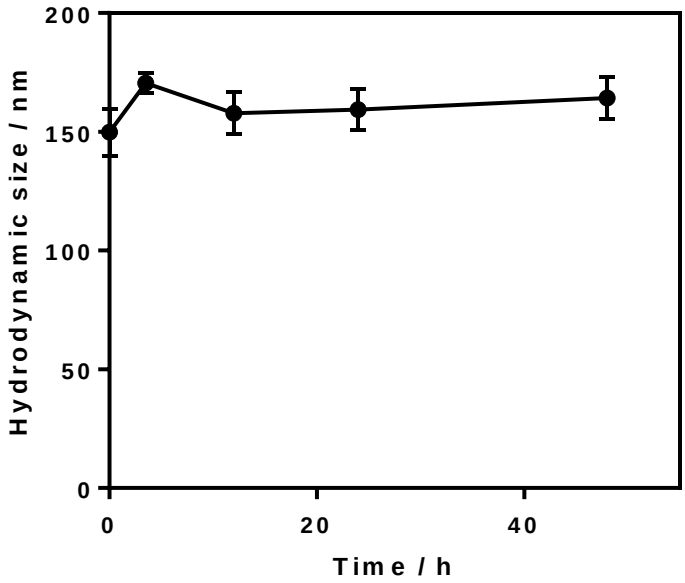


Figure S20. DLS hydrodynamic size distribution recorded for SNM-3 in FBS (10%) at different time intervals.

Cell study

Cell culture. Chang Liver cells were incubated in Dulbecco's Modified Eagle Medium (DMEM) containing FBS (10%) and penicillin/streptomycin (1%). HeLa cells were incubated in Dulbecco's Modified Eagle Medium (DMEM) containing FBS (10%) and penicillin/streptomycin (1%). A549 cells were incubated in RPMI-1640 medium containing FBS (10%) and penicillin/streptomycin (1%). MCF-7 cells were incubated in Modified Eagle Medium (MEM) containing FBS (10%) and penicillin/streptomycin (1%). Cells were incubated at 37 ℃ and 5% CO_2.

In vitro cell internalization and intracellular singlet oxygen detection. SNM-3 (10 μM) was incubated with HeLa cells at 37 ℃ for 2 h. Cells were washed three times with PBS, and stained with Lysotracker green for 15 min. The fluorescence images were recorded on a confocal laser scanning microscope. For intracellular $^1O_2$ detection, HeLa cells were incubated with SNM-3 (10 μM), SNM-1 (10 μM) or TPP-OH (10 μM) for 4 h. Subsequently, medium was removed, and cells were washed by PBS for three times. Fresh medium containing DCF-DA (20 μM) was added and cells were incubated for another 30 min followed by thorough washing with PBS. Cells were imaged by fluorescence microscopy before or after laser irradiation (671 nm, 0.2 W/cm^2).

Cytotoxicity evaluation. The cytotoxicity of different formulations was determined by CCK-8 assay with different cell lines. In a typical dark cytotoxicity experiment, cells were seeded in 96-plate wells (4000 cells/well, n = 6) and cultured for 12 h. Then, cells were treated with fresh medium containing SNM-3 at various concentrations. After 24 h incubation, the medium was removed and CCK-8 solution (10 μL) and fresh medium (90 μL) were added to each well. After another 2 h incubation, the absorbance was determined at 450 nm by microplate reader (Biotek Synergy H1). The relative cell viability was calculated against the control group (cells treated with medium). In a typical combination therapy experiment, cells were seeded in 96-plate wells (4000 cells/well, n = 6) and cultured for 12 h. Then, the cells were treated with fresh medium containing SNM-3 at different concentrations. After 4 h incubation, cells were irradiated with laser (671 nm, 0.2 W/cm^2) for 5 min each well. After irradiation, cells were further incubated for 20 h. Then medium was removed, and CCK-8 solution (10 μL) and fresh medium (90 μL) were added to each well. After another 2 h incubation, the absorbance was determined at 450 nm by microplate reader. The relative cell viability was calculated against the control group.

Statistical analysis.

The statistical analyses were performed using unpaired two-tailed Student's t test. All statistical analysis was carried out by using GraphPad Prism 6 software.

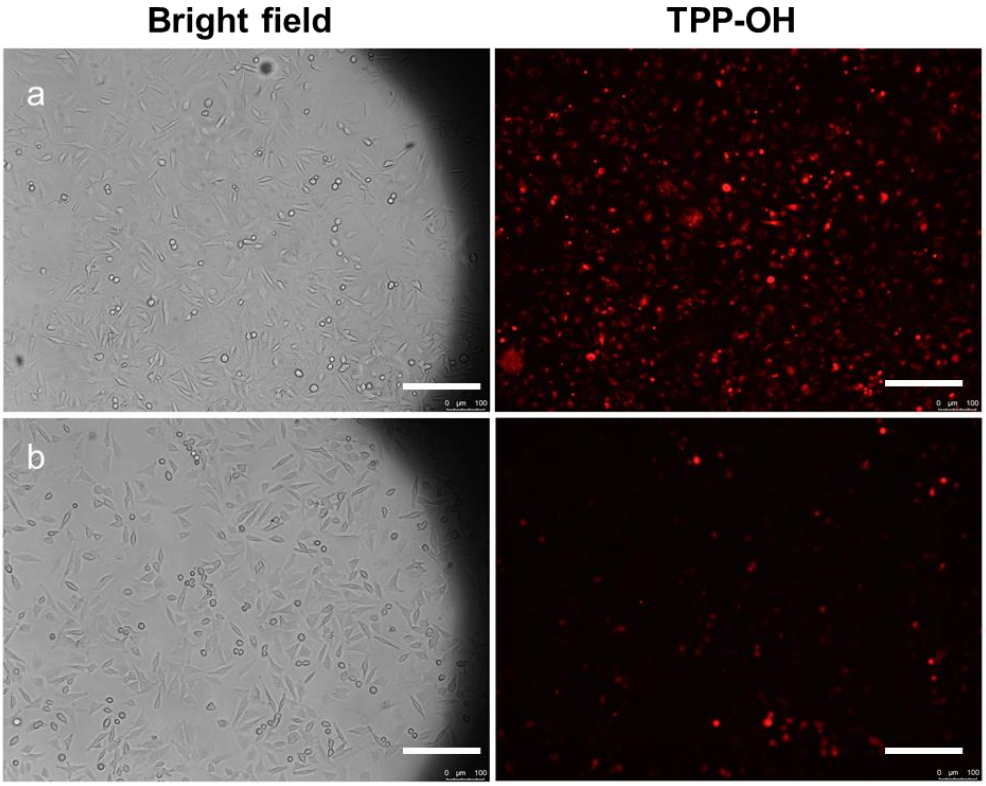


Figure S21. Fluorescence microscopic image recorded for HeLa cells incubated with (a) SNM-3 or (b) TPP-OH for 4 h. Scale bar: 200 μm.

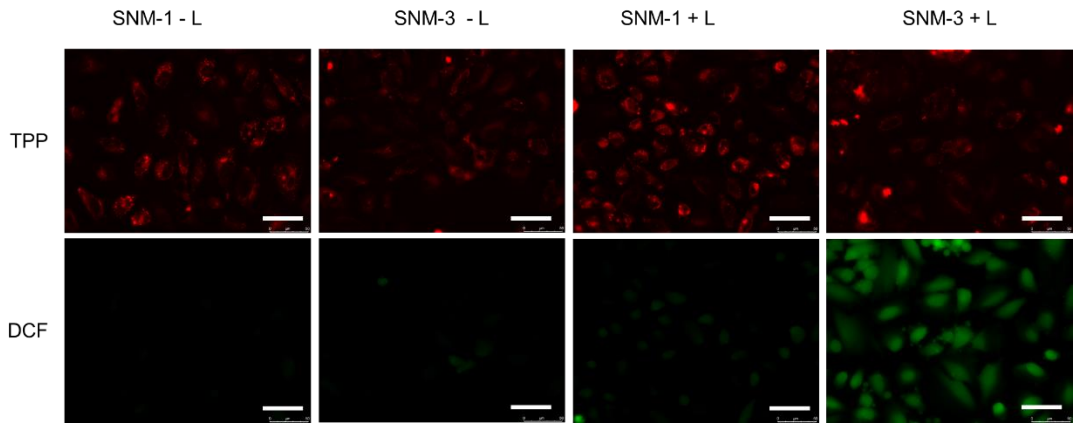


Figure S22. Fluorescence microscopic image recorded for HeLa cells incubated with SNM-3, SNM-1 (10 μM) for 4 h and stained with DCF-DA in the absence and presence of laser irradiation (671 nm. 0.2 W/cm^2, 3 min). Scale bar: 100 μm.

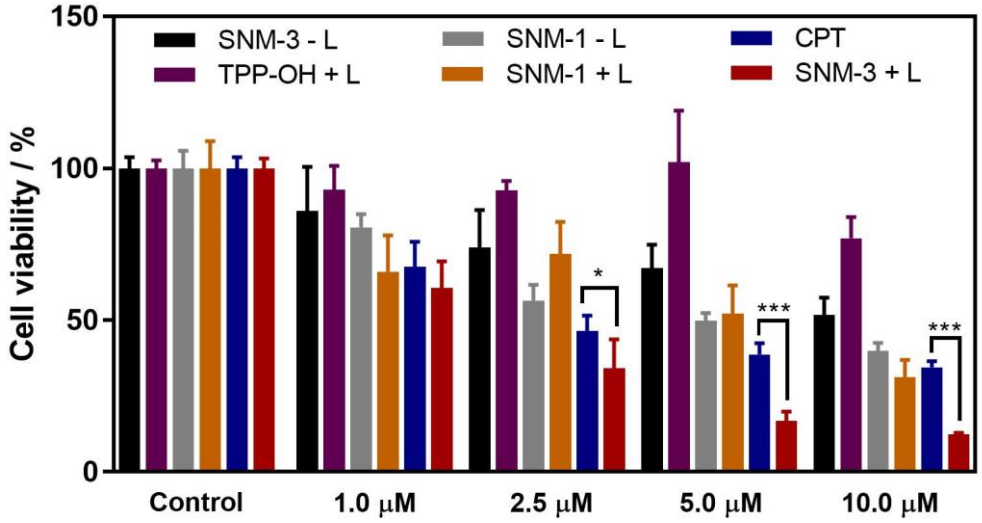


Figure S23. Photocytotoxicity and dark cytotoxicity of SNM-3, TPP-OH, SNM-1 and CPT to A549 cells (671 nm, 0.2 W/cm^2, 5 min per well). All cell viability is displayed as mean ± SD (n = 6, ***p < 0.001, *p < 0.05, Student's t test).

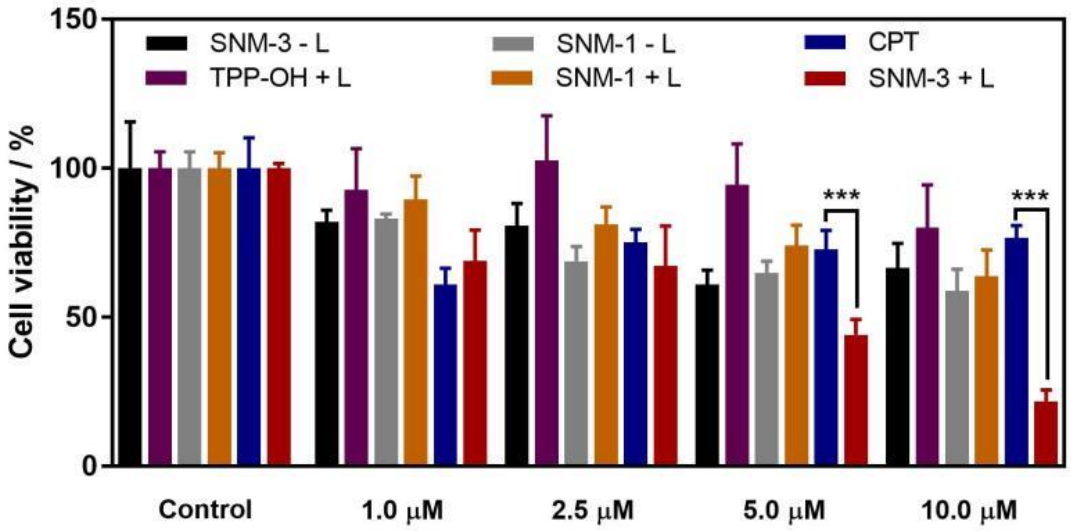


Figure S24. Photocytotoxicity and dark cytotoxicity of SNM-3, TPP-OH, SNM-1 and CPT to MCF-7 cells (671 nm, 0.2 W/cm^2, 5 min per well). All cell viability is displayed as mean ± SD (n = 6, ***p < 0.001, Student's t test).

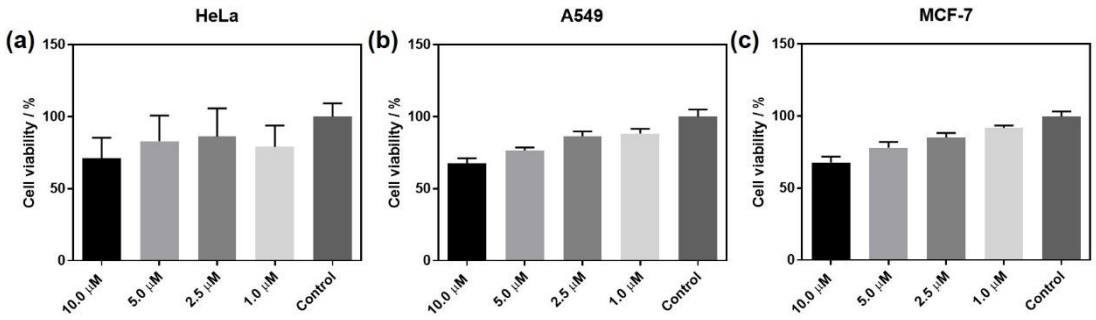


Figure S25. Dark cytotoxicity of CPT-mPEG against HeLa, A549 or MCF-7 cell line. Incubation time: 24 h.

References

1 W. Shao, X. Liu, G. Sun, X. Y. Hu, J. J. Zhu and L. Wang, *Chem. Commun.*, 2018, **54**, 9462–9465.

2 L. Xu, L. Liu, F. Liu, H. Cai and W. Zhang, *Polym. Chem.*, 2015, **6**, 2945–2954.