Electronic Supplementary information

Co-assembling FRET nanomedicine with self-indicating drug release

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Experimental Section

Materials and animals

Oleic acid (OA) was obtained from Sigma-Aldrich. 2,2-Dithiodiethanol and dicyclohexylcarbodiimide (DCC) were purchased from Alfa Aesar (MA, USA). 4-dimethylaminopyridine (DMAP) and triphosgene were purchased from Adamas Reagent (China). Camptothecin (CPT), curcumin (Cur) and Dithiothreitol (DTT) were purchased from MEILUN Biology Technology Co., LTD. (Dalian, China). The DSPE-mPEG$_{2000}$ was purchased from Lipoid GmbH (Ludwigshafen, Germany). All solvents used in this study were analytical grade.

Male BALB/c mice (6–8 weeks old) were purchased from the Laboratory Animal Center of Sichuan University (Chengdu, China). All animal procedure was conducted in compliance with the guidelines of Institutional Animal Care and Treatment Committee of Sichuan University, which oversees conformity with national law (Animal Welfare Act). Mice were humanely treated and all animal procedures were approved by the Institutional Animal Care and Treatment Committee of Sichuan University.

Synthesis of CPT-etcSS-OA, Cur-etcSS-OA and CPT-hec-OA

CPT-etcSS-OA and CPT-hec-OA were synthesized as described in our previous report [1].

For synthesis of Cur-etcSS-OA, the OA-etcSS-OH (100 mg) and DMAP (2.0 molar eq.) was dissolved in 10 ml anhydrous dichloromethane (DCM) at 0 °C under nitrogen. Triphosgene (0.35 molar eq.) in the anhydrous DCM was then added dropwise, and the solution was stirred at 0 °C for 10 min. Cur (1.0 molar equiv.) was then added into the solution, and reaction was performed at room temperature overnight. The solution was then evaporated to dryness, and Cur-etcSS-OA was purified by silica gel column chromatography (~yield 30 %).

Preparation of nanoaggregates of CPT-NAs, Cur-NAs and CPT-Cur-NAs

CPT-etcSS-OA nanoaggregates (CPT-NAs) and Cur-etcSS-OA nanoaggregates (Cur-NAs) were prepared according to the nanoprecipitation method. Briefly, CPT-etcSS-OA (or Cur-etcSS-OA) and DSPE-mPEG$_{2000}$ (10 % versus lipophilic prodrugs) was co-dissolved in the ethanol, which were dispersed dropwise into distilled water under the vigorous agitation.

Cur-etcSS-OA was co-assembled with CPT-etcSS-OA and CPT-hec-OA using the above procedure, thus to obtain the CPT-Cur-NAs of CPT-etcSS-OA and CPT-hec-OA, respectively.

Characterization of nanoaggregates

The size and size distribution were measured using Zetasizer (Nano-ZS90, Malvern, England) at 25 °C. Transmission electron microscopy (TEM, JEM-1200EX, Japan) was utilized to observe the morphology of nanoaggregates. Samples were stained with 2 % uranyl acetate. Colloidal stability of CPT-Cur-NAs was investigated by measuring their particle sizes in water stored at 37 °C for 48 h.

FRET measurements

The emission spectra of the CPT-NAs, Cur-NAs, mixed solution of CPT-NAs and Cur-NAs,

and CPT-Cur-NAs and were determined by a fluorescence spectrometer (Thermo varioskan flash, Thermo scientific). For FRET measurements, nanoaggregates were loaded in a 96-well plate, and was excited at 362 nm with the band-pass of 5 nm. The emission spectra were recorded in 2 nm intervals from 380 to 600 nm.

Intracellular monitoring/imaging drug release using FRET

Colorectal cancer cells (CT26) were purchased from American Type Culture Collection (Rockville, MD, USA). CT26 cells were maintained in RPMI 1640 containing 10 % FBS, penicillin (100 units/ml) and streptomycin (100 μg/ml) in a humidified atmosphere of 5 % CO2 at 37 °C. To monitor the intracellular drug release from CPT-OA conjugates, CT-26 (10^5) were seeded into 35 mm microscopy dishes, incubated at 37 °C for 24 h, and then incubated with CPT-Cur-NAs of CPT-etcSS-OA and CPT-hec-OA at an equivalent CPT concentration of 5 μg/ml for 5 min, 30 min, 2 h and 6 h, respectively. Cells were then rinsed with PBS five times and fixed in 4 % paraformaldehyde. The fixed cells were examined using a confocal laser scanning microscope (CLSM, Nikon, ECLIPSE, Ti2) with excitation at 405 nm to capture CPT fluorescence.

Intracellular drug release detected by HPLC

To quantitatively determine the CPT release from the prodrug within cells, the CT 26 cells were exposed to the CPT-NAs of CPT-etcSS-OA and CPT-hec-OA at CPT equivalent doses of 5 μg/ml at 37 °C for 6 h. The medium was then removed and cells were washed with cold PBS five times. 300 μl methanol (containing 1 % acetic acid) was added to each well. The cell lysate was centrifuged at 10,000 rpm for 10 min, and 20 μl of the supernatant was injected to HPLC system for analysis.

Cytotoxicity Assay

The cell viability was assessed by MTT assay. Briefly, CT26 cells were seeded in a 96-well plate at a density of approximate 5000 cells per well. After 24 h of growth, the medium was exchanged for the medium that contained CPT-Cur-NAs of CPT-etcSS-OA and CPT-hec-OA at various concentrations. The cell was further incubated for 48 h, and these without any treatment were utilized as control.

Monitoring drug release in the tissue homogenates using FRET

A subcutaneous model of colorectal cancer was established by subcutaneously injecting CT26 cell (2×10^6 cells per 100 μl) into the right axillary flank region of male BALB/C mice. When the tumor grew to around 500 mm^3, the mice were sacrificed. Blood was collected and centrifuged to obtain plasma, and the tumor, spleen, heart, lung, liver and kidney were excised. Organs were rinsed in normal saline and dried with the tissue paper to remove excess fluid. All these plasma and tissue samples were stored at 80 °C.

Mouse tissues (100 mg) were weighed, mixed with 300 μl saline, then homogenized using a tissue homogenizer. Then, CPT-Cur-NAs of CPT-etcSS-OA and CPT-hec-OA were supplemented with 100 μl of plasma and various tissue homogenates at the final CPT equivalent concentration of 10 μg/ml in the a 96-well plate. The plate was incubated at 37 °C and measured at the given time interval using the FRET measurement as described above.

Supporting Figures

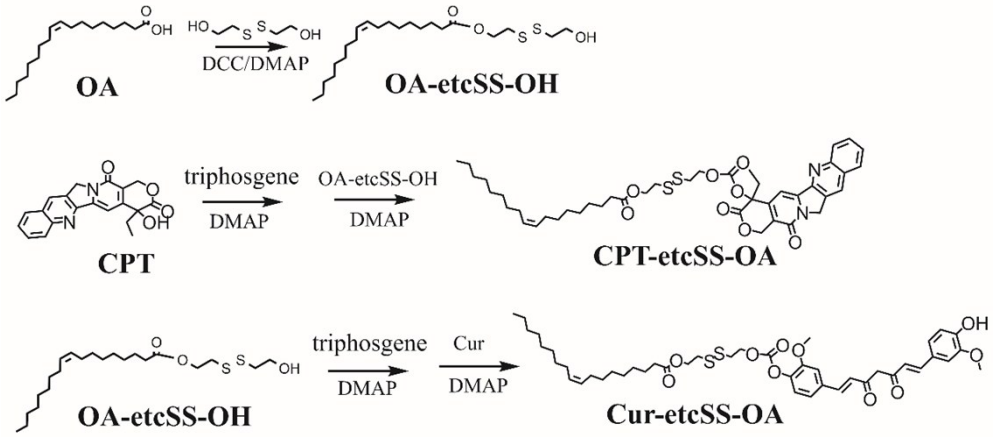

Fig.S1 Synthesis of CPT-etcSS-OA and Cur-etcSS-OA.

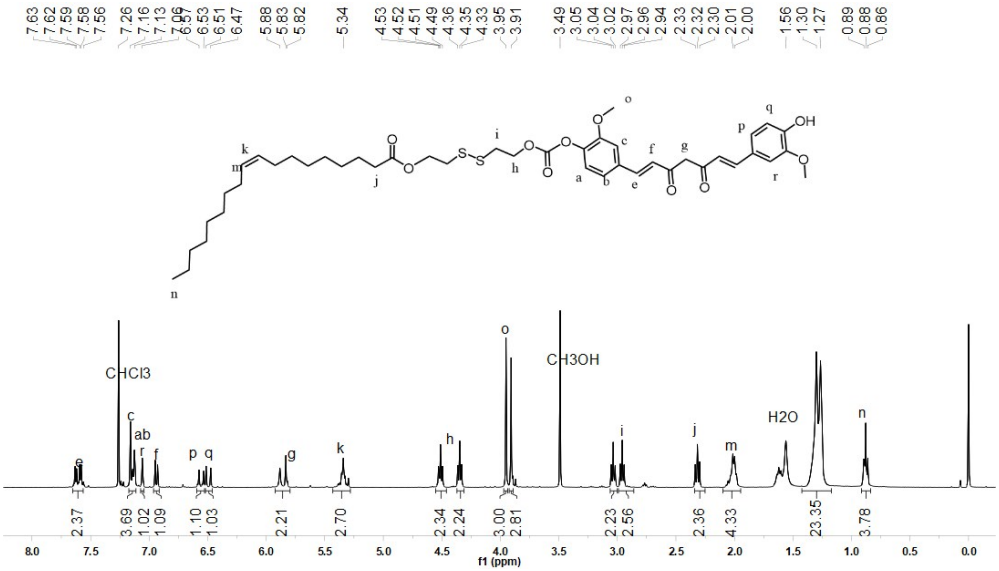

Fig.S2 ^{1}H NMR spectrum of the Cur-etcSS-OA

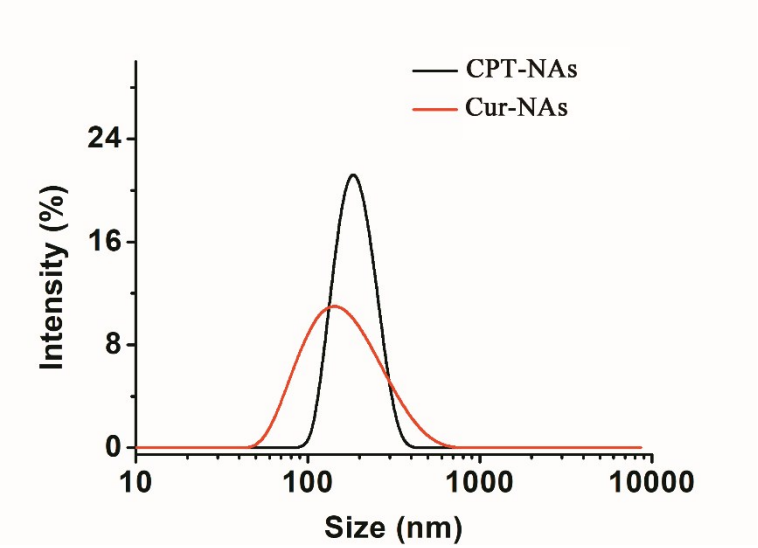

Fig.S3 Size distribution of CPT-NAs and Cur-NAs.

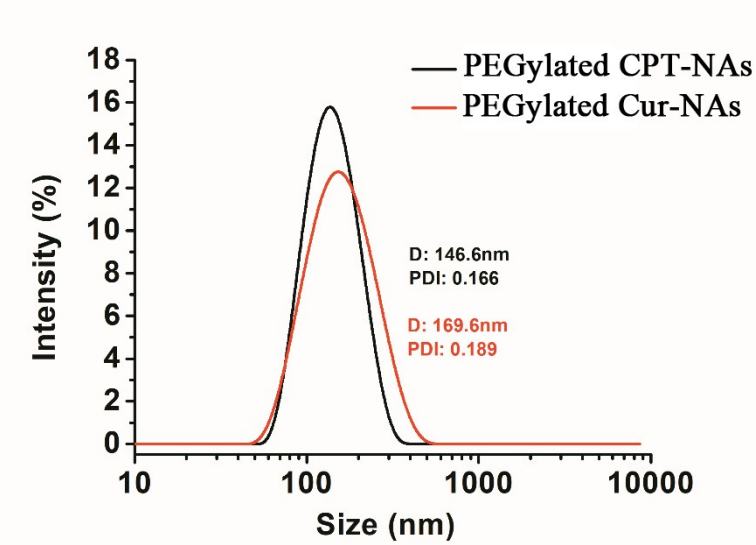

Fig.S4 Size distribution of PEGylated CPT-NAs and Cur-NAs.

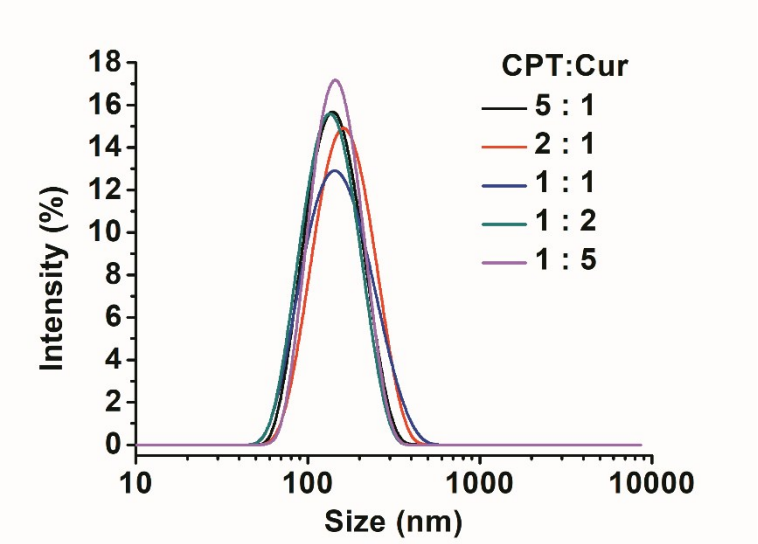

Fig.S5 Size distribution of CPT-etcSS-OA/Cur-etcSS-OA nanoaggregates (CPT-Cur-NAs) at various CPT-etcSS-OA/Cur-etcSS-OA ratios (mole/mole).

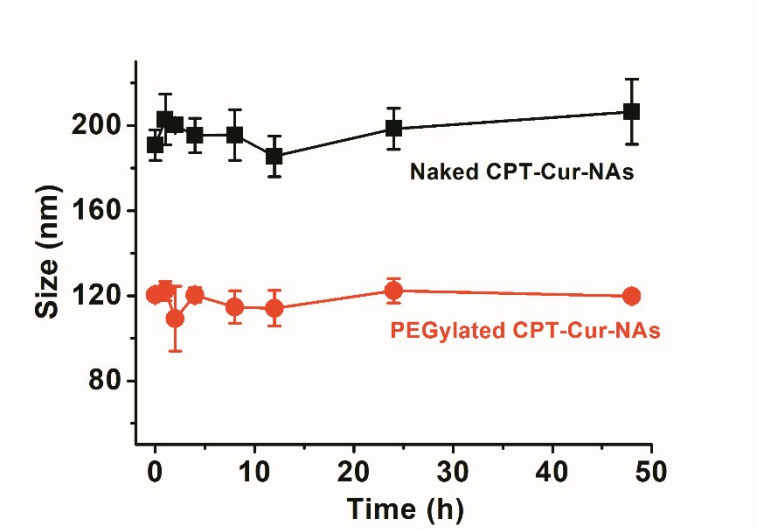

Fig.S6 Particle size of naked CPT-Cur-NAs and PEGylated CPT-Cur-NAs prepared at CPT /Cur ratio of 1/2 in water stored at 37 °C for 48 h, [means ± SD, n = 3].

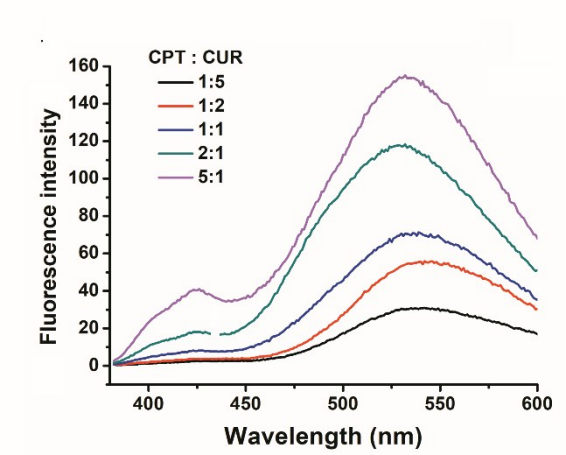

Fig.S7 Effects of the CPT-etcSS-OA/Cur-etcSS-OA ratio on the emission spectra of CPT-Cur-NAs excited at 362 nm.

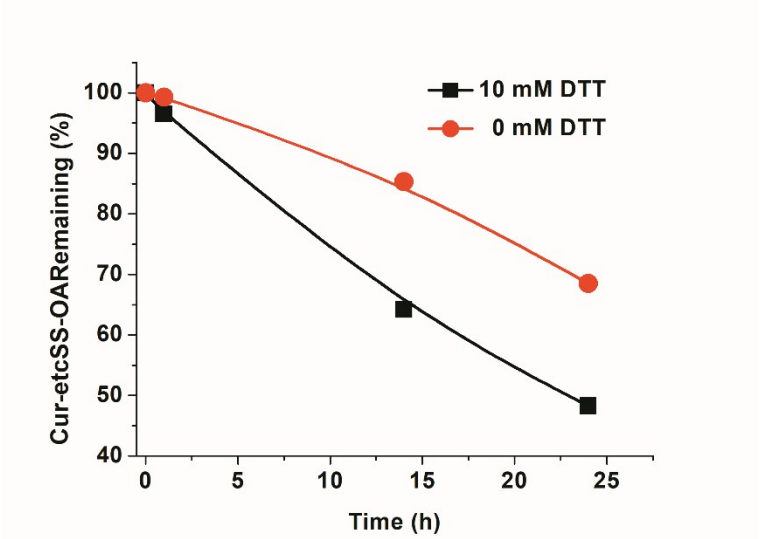

Fig.S8 Degradation of Cur-etcSS-OA with or without 10 mM DTT in 10 mM PB (pH 7.4) at 37 °C.

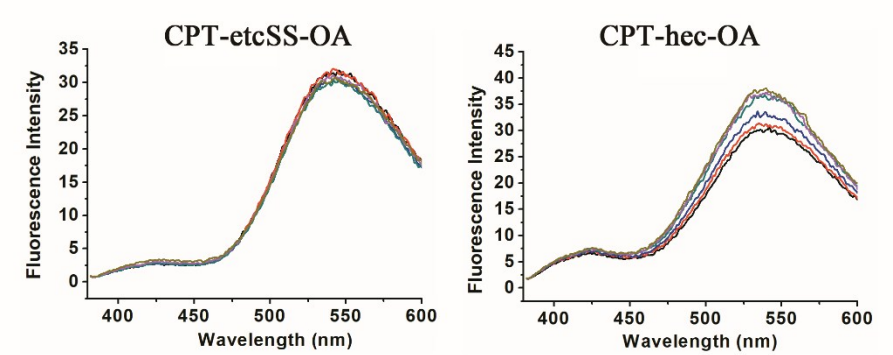

Fig.S9 kinetic change of emission spectra excited at 362 nm in 10 mM PB (pH 7.4) at 37 °C for CPT-Cur-NAs of CPT-etcSS-OA and CPT-hec-OA.

Reference

1. Y. Li, T. Kang, Y. Wu, Y. Chen, J. Zhu and M. Gou, *Chem. Commun.*, 2018, **54**, 1996-1999.