Electronic Supplementary Information (ESI)

Enzyme-Responsive Fluorescent Camptothecin Prodrug/Polysaccharide Supramolecular Assembly for Targeted Cellular Imaging and in Situ Controlled Drug Release

Yu-Hui Zhang,^{ab} Ying-Ming Zhang,^b Xianliang Sheng,^a Jie Wang^a and Yu Liu*^{bc}

^aCollege of Science, Inner Mongolia Agricultural University, Hohhot 010018, P. R. China.

^bDepartment of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, P. R. China.

^cCollaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin 300072, P. R. China.

*Address correspondence to yuliu@nankai.edu.cn

Experimental Section

General Methods. All chemicals were reagent grade unless noted. Annexin V-APC/PI apoptosis detection kit was purchased from KeyGen BioTECH and used as received. HACD was synthesized according to our reported method,¹ and AdaCPT was synthesized according to the reported method.² NIH3T3 mouse embryonic fibroblast cell line and HCT-116 human colon cancer cell line were obtained from China Infrastructure of Cell Line Resource. The UV/vis spectra were recorded in a conventional quartz cell (light path 10 mm) on a Hitachi U-2900 UV/vis spectrophotometer. Fluorescence spectra were recorded in a conventional quartz cell $(10 \times 10 \times 45 \text{ mm})$ on a Horiba Fluorolog®-MAX 4 fluorescence spectrometer. Highresolution transmission electron microscope (HR-TEM) images were obtained on a FEI Tecnai G² F20 transmission electron microscope. The samples were prepared by placing a drop of solution onto a carbon-coated copper grid and air-dried. The SEM images were performed on a JSM-6700F scanning electronic microscope. The DLS and zeta potentials were recorded on Malvern Zetasizer Nano ZS90 (Malvern Instruments Ltd., Worcestershire, UK). The fluorescent confocal images were recorded on a Leica TCS SP8 fluorescence microscope. NMR spectra were recorded on Bruker AV500 instruments. ESI-MS spectrum was measured using an Agilent 6520 Q-TOF-MS in positive-ion mode. Flow cytometry analyses were performed on Beckman CytoFLEX flow cytometer.

Synthesis of compound 2. 1-Adamantanecarbonyl chloride (500 mg, 2.52 mmol) was added to a solution of 2-hydroxyethyl disulfide (776 mg, 5.03 mmol) and TEA (382

mg, 3.77 mmol) in anhydrous DCM, which was stirred at 25 °C for 24 h under argon. The reaction mixture was washed with H₂O, saturated NaCl solution, and dried with anhydrous Na₂SO₄, filtrated and concentrated under vaccum. The obtained residue was purified by chromatography to give 2 as a yellow oil (280 mg, 35.2%). ¹H NMR (500 MHz, CDCl₃): 4.33 (t, J = 6.6 Hz, 2H), 3.89 (t, J = 5.7 Hz, 2H), 2.90 (m, 4H), 2.01 (s, 3H), 1.89 (d, J = 2.7 Hz, 6H), 1.71 (m, 6H).

Synthesis of reference compound ADACPT. Triphosgene (30 mg, 0.1 mmol) was added into the mixture of camptothecin (100 mg, 0.287 mmol) and DMAP (110 mg, 0.9 mmol) in dry DCM (10 mL) at 0 °C, the mixture was stir for 30 min at 25 °C, then the solution of compound 2 (84 mg, 0.264 mmol) in dry DCM was dropwise added into the mixture. The solution was stirred under room temperature for 12 h under argon. The reaction mixture was washed with H₂O, saturated NaCl solution, and dried with anhydrous Na₂SO₄, filtrated and concentrated under vaccum. The obtained residue was purified by chromatography to give ADACPT as pale yellow solid (120 mg, 65.6%).¹H NMR (500 MHz, CDCl₃): 8.40 (s, 1H), 8.23 (d, J = 8.6 Hz, 1H), 7.95 (d, J = 7.8 Hz, 1H), 7.84 (m, 1H), 7.68 (m, 1H), 7.34 (s, 1H), 5.70 (d, J = 17.2 Hz, 1H), 5.39 (d, J = 17.2 Hz, 1H), 5.31 (s, 2H), 4.37 (m, 2H), 4.24 (t, J = 6.5 Hz, 2H), 2.93 (m, 2H), 2.89 (t, J = 6.4 Hz, 2H), 2.25 (m, 2H), 1.99 (s, 3H), 1.84 (d, J = 2.7 Hz, 6H), 1.68 (d, J = 8.2 Hz, 6H), 1.00 (t, J = 7.5 Hz, 3H). HR-MS (MALDI-TOF): *m/z* calcd for (C₃₆H₃₈N₂O₈+H⁺), 691.2142, found: 691.2141.

Preparation of HACD-AdaCPT supramolecular polysaccharide assembly. AdaCPT was dissolved in DMSO to prepare a stock solution with the concentration of 12 mM. HACD was dissolved in deionized water to obtain a stock solution with the [CD] concentration of 3 mM. The stock solution of HACD was diluted 5 folds to 0.6 mM. Upon sonication, AdaCPT stock solution (50 μ L) was slowly added into the diluted host solution (950 μ L) over 5 min. The resulting HACD-AdaCPT solution was stored at 4°C. The loading efficiency of AdaCPT in assembly was calculated by UV/vis standard curve of AdaCPT with absorption at 375nm after assembly solution dialyzed against excess amount of deionized water for 1 h.

Reduction-sensitivity of HACD-AdaCPT assembly. The reduction responsive of HACD-AdaCPT supramolecular assembly was analyzed by Uv/vis and fluorescence spectrum. DL-dithiothreitol (DTT) was chosen as the model reductant. For Uv/vis spectrum, HACD-AdaCPT supramolecular assembly (5 μ M) were treated with or without 5 mM DTT in PBS buffer (0.01 M, pH 7.4) at 37 °C for 2 h. For time-dependent fluorescence spectrum, HACD-AdaCPT supramolecular assembly (5 μ M) were treated with 5 mM DTT in PBS buffer (0.01 M, pH 7.4) at 37 °C, the spectrum was recorded from 0 to 80 min.

Cytotoxicity experiments. NIH3T3 mouse embryonic fibroblast cell line was cultured in Dulbecco's modified Eagle's medium (DMEM), HCT-116 human colon cancer cell line was cultured in the McCoy's 5A medium, both of the medium were supplemented with 10% fetal bovine serum (FBS). NIH3T3 cells and HCT-116 cells were seeded in 96-well plates (5×10^4 cells/mL, 100 µL per well) for 24 h, then the cells were incubated with HACD, CPT, AdaCPT, HACD-AdaCPT, HACD-AdaCPT + excess HA, ADACPT, HACD-ADACPT, ADAND, HACD-ADAND ([CPT] = 15 µM) for 48 h, respectively. The relative cellular viability was determined by MTT assay.

Furthermore, the IC50 values of CPT, AdaCPT and HACD-AdaCPT toward HCT-116 cell line were evaluated with MTT assay. HCT-116 cells were seeded in 96-well plates $(5 \times 10^4 \text{ cells/mL}, 100 \mu \text{L} \text{ per well})$ for 24 h, then the cells were incubated with CPT, AdaCPT and HACD-AdaCPT at different concentrations for 48 h. All data were presented as the mean ± standard deviation.

Fluorescent confocal imaging. NIH3T3 cells and HCT-116 cells were cultured in confocal dish (5×10^4 cells/mL, 1.5 mL per well) for 24 h. The cells incubated with HACD-AdaCPT for 24 h, and then washed with PBS buffer for three times. After that the cells were fixed with 4% paraformaldehyde for 15 min and observed by a confocal laser scanning microscope (blue channel at 450 nm, green channel at 515 nm). In order to evaluate the receptor mediated endocytosis, lysosomes were stained with Lysotracker Red at 200 nM for 30 min after incubation with cells for colocalizing polysaccharide assembly. To gain more insight into the targeting selectivity of HACD-AdaCPT polysaccharide assembly to HCT-116 cells, the cellular uptake of assembly with and without excess amount of HA was studied by confocal laser scanning microscopy.

Cellular uptake of HACD-AdaCPT supramolecular polysaccharide assembly. HCT-116 cells were cultured in confocal dish (5×10^4 cells/mL, 1.5 mL per well) for 24 h. The cells incubated with HACD-AdaCPT for 1 and 12 h, respectively. Then the cells were washed with PBS buffer for three times and fixed with 4% paraformaldehyde for 15 min. The samples were observed by a confocal laser scanning microscope (blue channel at 450 nm, green channel at 515 nm).

Cell apoptosis. HCT-116 cells were seeded in 6-well plates (4×10^5 cells/mL, 2 mL per well) for 24 h, then the cells were incubated with CPT, AdaCPT and HACD-AdaCPT ([CPT] = 4 μ M) for 24 h. For the measurement of apoptosis, cells were harvested and stained with Annexin V-APC/PI according to kit instructions. HCT-116 cells without the treatment were used as control.



Scheme S1 The synthetic routes of reference compound ADACPT.



Fig. S1 (a) Optical transmittance of HACD-AdaCPT polysaccharide assembly by increasing concentration from 0.025 to 10 μ M at 25 °C in water, (b) dependence of the optical transmittance at 625 nm for assembly.



Fig. S2 UV/vis absorbation of AdaCPT at 5, 7, 10, 15, 20 μ M in aqueous solution. Inset: Standard curve of AdaCPT with absorption at $\lambda = 375$ nm vs concentration.



Fig. S3 HACD-AdaCPT supramolecular polysaccharide assembly solution, A: newly prepared, B: stocked for months. (a) photography, (b) Tyndall effect.



Fig. S4 ESI-MS of HACD-AdaCPT supramolecular polysaccharide assembly after treated with DTT.



Fig. S5 Proposed reaction mechanism of AdaCPT with thiols under physiological conditions.



Fig. S6 (a) TEM image, (b) DLS result of HACD-AdaCPT supramolecular polysaccharide assembly treated with DTT.



Fig. S7 Colocalizing confocal fluorescence images of HCT-116 cells incubated with HACD-AdaCPT supramolecular polysaccharide assembly for 24 h. Lysomes were stained with Lysotracker Red.



Fig. S8 Confocal fluorescence images of HCT-116 cells incubated with HACD-AdaCPT supramolecular polysaccharide assembly for 24 h. (a) without, (b) with excess amount of HA.



Fig. S9 Confocal fluorescence images of HCT-116 cells incubated with HACD-AdaCPT supramolecular polysaccharide assembly at 1h and 12 h.



Fig. S11 ¹H NMR (500 MHz) spectrum of reference compound ADACPT in CDCl₃ at

25 °C.





Fig. S13 Relative cellular viability of HCT-116 cell lines in 48 h ([CPT] = 15μ M).



Fig. S14 ¹H NMR (500 MHz) spectrum of reference compound ADAND in CDCl₃ at 25 °C.



Fig. S15 The curves of HCT-116 cell inhibitory rate at different concentrations of (a) CPT, (b) AdaCPT and (c) HACD-AdaCPT.



Fig. S16 Flow cytometry analysis for apoptosis of HCT-116 cells induced by CPT, AdaCPT, and HACD-AdaCPT for 24 h ([CPT] = 4 μ M).

References

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