Supplemental information for

**Small molecular nanomedicines formed from camptothecin dimer containing disulfide bond**

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Experimental details

Materials. All starting materials were purchased from commercial suppliers and used without further purification unless otherwise noted. Solvents for chemical synthesis were purified by distillation.

Synthesis of CPT-s-s-CPT. It has been reported by J. Cheng’s group previously,\textsuperscript{1} so it is not described here.

Characterization of CPT-s-s-CPT. \textsuperscript{1}H NMR spectra was recorded on a Bruker AV400 M in CDCl\textsubscript{3} at 25 °C. Chemical shifts were given in parts per million from that of tetramethylsilane (TMS) as an internal reference.

Preparation of CPT nanoparticles (CPT-NPs). The CPT-NPs were prepared as follows: 1.45 mg of CPT-s-s-CPT was dissolved in 4 mL of N, N-dimethylformamide (DMF) and stirred for half an hour. Then the solution was added dropwise to 10 mL of deionized water. DMF and unself-assembled CPT-s-s-CPT were removed by dialysis against distilled water (MWCO=1000).

The concentration of CPT-NPs was determined by the UV absorbance at 364 nm for CPT-s-s-CPT, according to the standard calibration curve of CPT-s-s-CPT in DMF. The solution of CPT-NPs was diluted 10 times with DMF, then was measured by UV-vis spectrophotometer performed on Shimadzu UV-2450 PC.

CPT-NPs characterization. Size distribution of the CPT-NPs was determined by dynamic light scattering (DLS) with a vertically polarized He-Ne laser (DAWN EOS, Wyatt Technology, U.S.A.). The scattering angle was fixed at 90° and the measurement was carried out at 25 °C. The morphology of the CPT-NPs was
measured by Transmission Electron Microscopy (TEM) performed on a JEOL JEM-1011 electron microscope operating at an acceleration voltage of 100 kV. To prepare the specimen for TEM, a drop of nanoparticles solution was deposited onto a copper grid with a carbon coating. The specimen was air-dried and measured at room temperature. It was also measured by Scanning Electron Microscopy performed on JEOL JXA-840 under an accelerating voltage of 15 kV.

**Cell culture.** The human cervical cancer cell line HeLa and human hepatocarcinoma cell line HepG2 were routinely grown in Dulbecco's Modified Eagle Medium (DMEM) (Gibco) containing 10% FBS (Hyclone). Cells were cultured in a humidified incubator at 37 °C with 5% CO₂.

**Confocal laser scanning microscopy (CLSM).** HeLa and HepG2 cells were seeded on the coverslip in 6-well plates at a density of 5 × 10⁵ cells per well in 1 mL of DMEM medium and incubated in a humidified 5% CO₂ atmosphere for 24 h. For cellular internalization observation, cells were incubated with CPT-NPs at the concentration of 20 μg mL⁻¹ in fresh culture medium. After incubation for 0.5 h and 2 h at 37 °C, cells were washed twice with ice-cold PBS and fixed with fresh 4% (w/v) paraformaldehyde for 15 min at room temperature. The cellular localization was visualized under a confocal laser scanning microscope (Carl Zeiss LSM 780).

**In vitro cytotoxicity.** The cytotoxicity of CPT-NPs was measured via MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Briefly, HeLa and HepG2 cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 2×10³ cells/well and incubated in DMEM for 24 h. The medium was then
replaced with CPT-NPs at various concentrations from 0.0005 to 5 μg/mL. At the designated time intervals (48 h), 20 μL of MTT solution in PBS with the concentration of 5 mg/mL was added and the plate was incubated for another 4 h at 37 °C. After that, the medium containing MTT was removed and 150 μL of DMSO was added to each well to dissolve the MTT formazan crystals. Finally, the plates were shaken for 3 min, and the absorbance of formazan product was measured at 490 nm by a microplate reader (BioTek, EXL808). Data are presented as means ± SD (standard deviation) (n=4).

The reduction-responsiveness of disulfide-based nanomedicines was conducted as follows: HeLa cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 2×10^3 cells/well and incubated in DMEM for 24 h. GSH with the final concentration of 10 mM was added and cultured with cells for 4 h. Then the medium was removed and the 96-well plates were washed two times, followed by addition of fresh medium and CPT-NPs at various concentrations from 0.0005 to 5 μg/mL. Subsequently cells were further incubated for 48 h, and cell viability was determined by MTT assay. Cells without GSH treatment were used as control.

**Statistics.** All experiments were performed at least three times and all results are expressed as mean ± SD.
Fig. S1 The UV-vis absorption standard curve of CPT-s-s-CPT in DMF.

Fig. S2 The picture of CPT (a), CPT-s-s-CPT (b) and CPT-NPs (c) in water.
Fig. S3 SEM image of CPT-NPs.

Fig. S4 change of size and size distribution of CPT-NPs with different times determined by DLS.
Fig. S5 The cell uptake of CPT-NPs. All scale bars stand for 20 μm.