Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Glutathione- and pH-Responsive Nonporous Silica Prodrug Nanoparticles for Controlled Release and Cancer Therapy

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Methods

Synthesis and characterization of stimuli-responsive silane prodrug (SSP) monomer of CPT (SSP-CPT). Synthesis of BHD-CPT: The synthetic route of SSP-CPT monomer is depicted in Figure S1, and the typical procedures was performed similarly to the previously published procedure. [S1] Camptothecin (CPT, 376.2 mg, 1.08 mmol) and DMAP (420.6 mg, 3.42 mmol) were suspended in methylene chloride (DCM, 30 mL) under argon atmosphere. Then triphosgene (BTC, 123 mg, 0.396 mmol) was added and the mixture was stirred for 30 min at room temperature. Bis(2-hydroxyethyl) disulfide (BHD, 830 mg, 5.4 mmol, in 4.0 mL THF) was added dropwise via a rubber septum using a gastight syringe. The reaction was stirred overnight during which time a white precipitate formed. After filtration to remove the white precipitate, and the reaction mixture was diluted with EtOAc (300 mL) and washed once with water (100 mL), twice with 1.0 M HCl (50 mL), and once with brine (100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated on a rotary evaporator. The solid residue was purified by column chromatography (100% EtOAc, TLC R_f = 0.5, visualize under UV light) to give BHD-CPT, a yellow solid (256.6 mg, yield: 45 %). ^{1}H NMR of BHD-CPT was shown in Figure S2. ^{1}H NMR (300 MHz, $CDCl_3$, δ): δ = 8.47 (s, 1H), 8.35-8.25 (d, 1H), 7.97 (d, 1H), 7.92–7.85 (m, 1H), 7.79 – 7.68 (m, 1H), 7.57 (s, 1H), 5.80 – 6.68 (m, 1H), 5.45 –5.3 (m, 3H), 4.40 (m, 2H), 3.91 (m, 2H), 3.05 – 2.85 (m, 4H), 2.35 – 2.11 (m, 2H), 1.08 – 0.98 (m, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, δ) 167.04, 156.45, 152.81, 152.13, 147.84, 146.21, 144.75, 131.54, 130.39, 129.67, 128.95, 128.45, 127.95, 127.70, 119.18, 94.38, 77.88, 66.45, 64.13, 59.56, 50.25, 41.12, 22.38, 13.87, 7.55; MS (ESI): calcd. for $C_{25}H_{24}N_2O_7S_2$, $[M+H]^+$ m/z 528.10, found 528.93.

Synthesis of SSP-CPT: Typical procedures for the synthesis of SSP-CPT are as follows: BHD-CPT (106 mg, 0.2 mmol) and dibutyltin dilaurate (DBTDL, 2 uL) were dissolved in 15 mL $CHCl_3$ at 60 ^{o}C under argon atmosphere, and then the 3-(triethoxysilyl)propyl isocyanate (75 mg, 0.3 mmol) in 2 mL $CHCl_3$ was added and refluxed for 6 h, and cooled to room

temperature. After removing the solvent $CHCl_3$, the crude product was purified by preparative TLC (CH_2Cl_2/MeOH = 100/1) to give SSP-CPT, a yellow solid (81.2 mg, yield: 52.2 %). ^{1}H NMR of SSP-CPT was shown in Figure S3. ^{1}H NMR (300 MHz, $CDCl_3$, δ): δ = 8.42 (s, 1H), 8.29-8.16 (d, 1H), 7.96 (d, 1H), 7.90–7.80 (m, 1H), 7.72 – 7.62 (m, 1H), 7.35 (s, 1H), 5.81–6.67 (m, 1H), 5.45–5.28 (m, 3H), 4.38 (m, 2H), 4.25–4.03 (m, 2H), 3.88–2.69 (m, 6H), 3.15 (m, 2H), 2.99–2.77 (m, 4H), 2.38 – 2.13 (m, 2H), 1.54 – 1.68 (m, 2H), 1.31 – 1.21 (m, 9H), 1.08 – 0.97 (m, 3H), 0.68 – 0.55 (m, 2H). ^{13} C NMR (100 MHz, DMSO-d_6, δ) 172.40, 166.98, 156.46, 152.76, 152.16, 147.87, 146.23, 144.70, 131.56, 130.38, 129.76, 128.96, 128.47, 127.98, 127.69, 119.16, 94.32, 77.86, 72.34, 66.41, 64.86, 59.40, 57.65, 55.99, 50.17, 41.11, 30.30, 22.89, 18.51, 15.11, 7.72; MS (ESI): calcd. for $C_{35}H_{46}N_3O_{11}S_2Si$ $[M+H]^+$ m/z 776.24, found 775.69.

Synthesis and characterization of SSP monomer of DOX (SSP-DOX). Synthesis of MABH: The synthetic route of SSP-DOX monomer is depicted in Figure S4. Firstly, the hydrazine bond monomer, MABH was prepared using tert-butyl carbazate and methacryloyl chloride according to our previous reports.[S2] Typically, to an anhydrous dichloromethane (DCM) solution (75 mL) of tert-butyl carbazate (3.96 g, 30 mmol) and triethylamine (5.46 mL, 36.0 mmol) at 0 ℃ were added dropwise an anhydrous DCM solution (30 mL) of methacryloyl chloride (3.07 mL, 30 mmol) simultaneously. The reaction was stirred at 25 oC for 12 h after completion of addition. The solution was then filtered to remove the salt, and the crude product was recovered by evaporation of DCM. The product was denoted as MABH and purified by silica column chromatography. (Eluent: n-hexane/EA = 80/20). Then, the product was concentrated and dried in vacuo. (4.8 g, yield: 80.0 %) ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.22, 6.96 (s, 2H, -NH), 5.42, 6.07(s, 2H, CH_2=), 1.96 (q, 3H, -CH_3), 1.45-1.53 (q, 9H, -$(CH_3)_3$). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 167.8, 155.9, 137.6, 121.5, 81.7, 28.29, 18.36. MS (ESI): calcd. for $C_9H_{16}N_2O_3$ $[M+Na]^+$ m/z 223.11, found 223.00.

Synthesis of BH-DOX: The BH-DOX was prepared similarly as previously reported.[S3] The resulting MABH (500 mg, 2.5 mmol) were dissolved in a mixture solution of DCM/TFA (v/v, 1:1) Reaction mixture was allowed stirring for 30 min at room temperature, and then the reaction mixture was concentrated and the excess TFA was removed by co-evaporated three times with toluene. Then the resulting deprotection product (60 mg, 0.3 mmol) and DOX•HCl (60 mg, 0.1 mmol) were dissolved in 10 mL of anhydrous methanol. Trifluoroacetic acid (3 μL) was added to the reaction mixture. The reaction mixture was stirred at room temperature for 48 h under dark, the final BH-DOX was isolated by adding excess acetonitrile. After filtration, and drying in vacuo to give BH-DOX, a dark red solid (40 mg, yield: 64 %). ^{1}H NMR of BH-DOX was shown in Figure S5. ^{1}H NMR (300 MHz, DMSO-d_6, δ): 11.8 (s, 1H), 7.98−7.63 (m, 3H), 5.80 (s, 1H), 5.53 (s, 1H), 5.47 (m, 1H), 5.07 (t, l H), 4.59 (d, 2H), 4.17 (m, lH), 3.91 (s, 3H), 3.57−3.55 (m, 1H), 3.16 (s, 1H), 2.23−2.21 (m, 2H), 2.13−2.15 (m, 2H), 1.99-1.87 (m, 3H), 1.76-1.35 (m, 3H), 1.21−1.14 (m, 3H). FTIR (KBr, cm^{-1}): ν = 3406.10, 1615.77, 1580.96, 1410.18, 1384.30, 1283.82, 1209.70, 1114.12, 1011.51, 986.17, 799.78; MS (ESI): calcd. for $C_{31}H_{35}N_3O_{11}$ $[M+H]^+$ m/z 626.23, found 625.83.

Synthesis of SSP-DOX: The resulting BH-DOX (30 mg, 0.05 mmol) was dissolved in anhydrous DMF (1.0 mL), and then TEA (18 μL, 0.14 mmol) and (3-mercaptopropyl) trimethoxysilane (13 μL, 0. 07 mmol) were added. The reaction mixture was stirred at room temperature for 12 h. After the solvent was evaporated in vacuum, the resulting rude product was purified by repeatedly precipitation into acetonitrile from methanol solution to give SSP-DOX, a dark red solid (20 mg, yield: 48.6 %). ^{1}H NMR of SSP-DOX was shown in Figure S6. ^{1}H NMR (300 MHz, DMSO-d_6, δ): 7.99−7.65 (m, 3H), 5.47 (m, 1H), 5.30 (t, lH), 4.59 (d, 2H), 4.17 (m, lH), 3.98 (s, 3H), 3.47−3.45 (m, 1H), 3.47 (m, 9H), 3.18 (s, 1H), 3.05−3.01 (m, 2H), 2.69 (t, 2H), 2.23−2.21 (m, 2H), 2.13−2.15 (m, 2H), 1.92-1.81 (m, 2H), 1.68 (q, 2H), 1.19−1.12 (m, 6H), 0.75 (t, 2H); FTIR (KBr, cm^{-1}): ν = 3399.67, 2934.13, 1615.54, 1579.30, 1445.44, 1408.93, 1283.33, 1209.07, 1113.93, 1067.65, 1011.85, 986.27,

872.27, 793.19, 763.25, 594.32; MS (ESI): calcd. for $C_{37}H_{51}N_3O_{14}SSi$ $[M+H]^+$ m/z 822.29, found 822.60.

The stability of silica prodrug NPs under physiological conditions: The stability of prodrug NPs was studied by the following method. Firstly, 0.2 mL of the prodrug NPs (CPT NPs-100 or DOX NPs-100) ethanol solutions were centrifuged at 14000 rpm for 10 min, and then the resulting prodrug NPs solid was dispersed in 1×PBS (pH 7.4) and then incubated at 37 °C with slight shake. After 120 h, a drop of sample solution was taken out and the general morphology and the sizes of prodrug NPs were measured by TEM. The TEM results were shown in Figure S11.

The stability of blank SiO_2 NPs as the control experiment: Similarly, 0.2 mL of the freshly prepared SiO_2 NPs-100 was centrifuged at 14000 rpm for 10 min, and then the resulting SiO_2 NPs solid was dispersed in 1×PBS (pH 7.4) containing 10 mM GSH and then incubated at 37 °C with slight shake. After for 72 h, a drop of sample solution was taken out and was measured by TEM as the control experiment. The TEM results were shown in Figure S12.

The morphology change of prodrug NPs under tumor microenvironment: The morphological change of prodrug NPs was studied by the following method. Firstly, 0.2 mL of the prodrug NPs (CPT NPs-100 or DOX NPs-100) ethanol solutions were centrifuged at 14000 rpm for 10 min, and then the resulting prodrug NPs solid was dispersed in 2 mL different buffer solutions (For CPT NPs-100 is in 1×PBS (pH 7.4) containing 10 mM GSH and for DOX NPs-100 is in pH 5.0 buffer solution), and then the sample solutions were incubated at 37 °C with slight shake. After 72 h, a drop of sample solution was taken out and the morphological change of prodrug NPs were observed by TEM. The TEM results were shown in Figure 3 b and c.

Supplementary References:

[S1] Z. Xu, D. Wang, S. Xu, X. Liu, X. Zhang, H. Zhang, *Chem. Asian J.* **2014**, *9*, 199-205.

[S2] Z. Xu, K. Zhang, C. Hou, D. Wang, X. Liu, X. Guan, X. Zhang, H. Zhang, *J. Mater. Chem. B* **2014**, *2*, 284-292.

[S3] D. Willner, P. Trail , S. Hofstead, H. King , S. Lasch, G. Braslawsky, R. Greenfi eld, T. Kaneko, R. Firestone, *Bioconjugate Chem.* **1993**, *4* , 521-527.

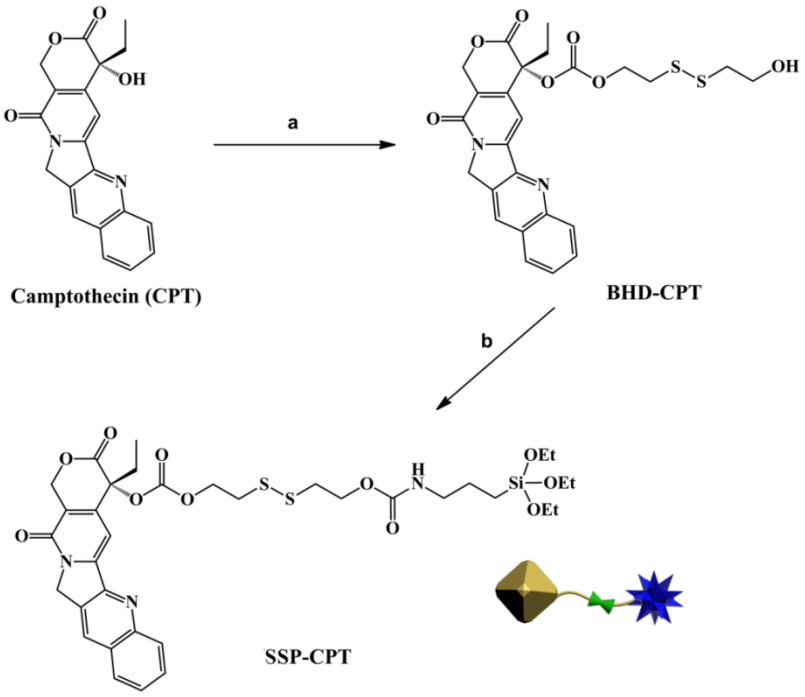

Figure S1. The synthesis route of SSP-CPT. (a) Triphosgene (BTC) and DMAP, in CH_2Cl_2, rt, argon atmosphere, 30 min, then bis(2-hydroxyethyl) disulfide (BHD) in THF added, rt, 12 h; (b) dibutyltin dilaurate, 3-(triethoxysilyl)propyl isocyanate in $CHCl_3$, 60 ^oC, argon atmosphere, refluxing for 6 h.

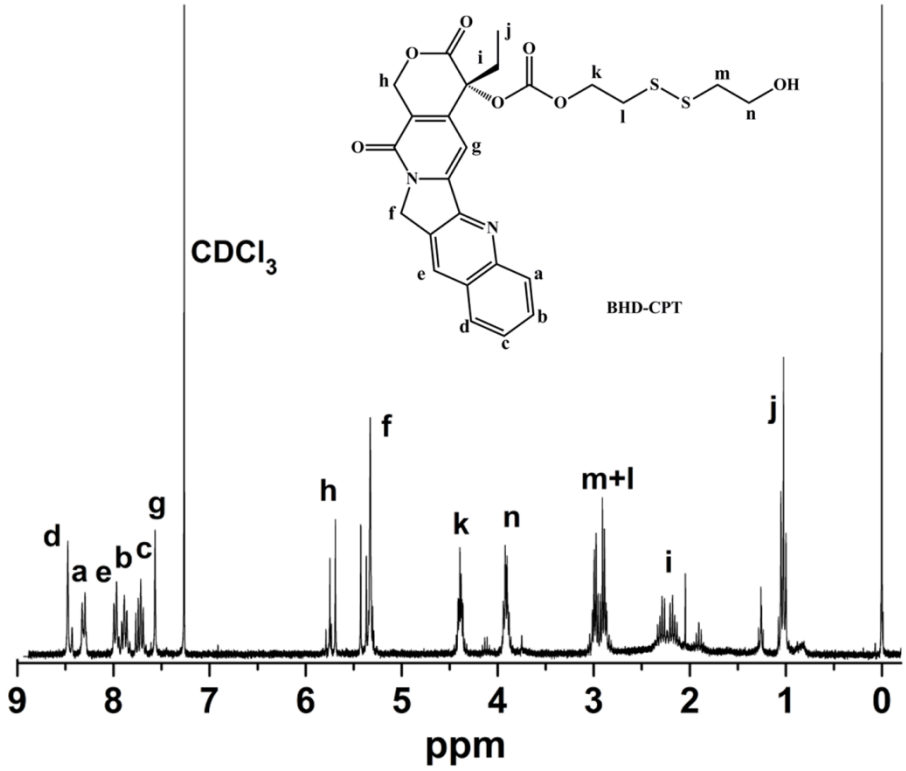

Figure S2. ^{1}H NMR spectrum (300 MHz, Chloroform-d) of BHD-CPT.

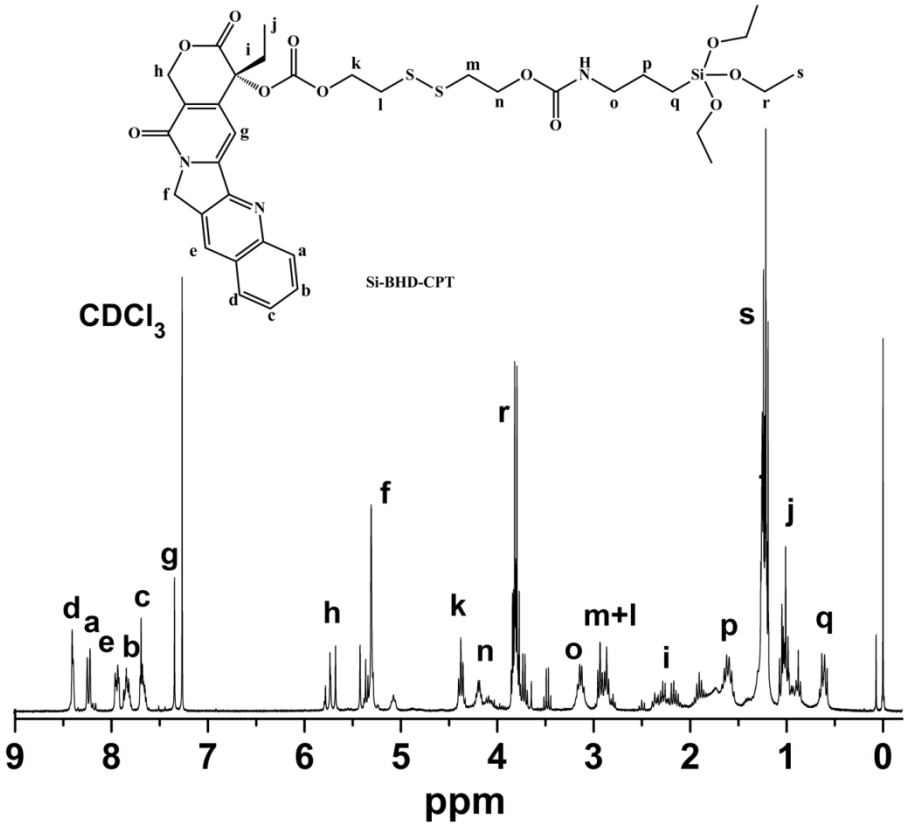

Figure S3. ^{1}H NMR spectrum (300 MHz, Chloroform-d) of SSP-CPT.

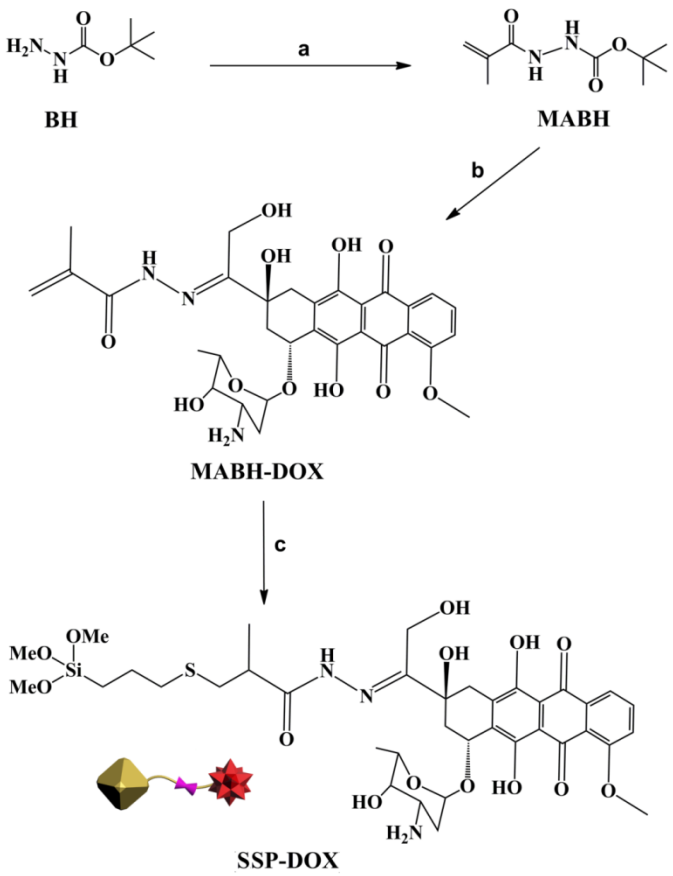

Figure S4. The synthesis route of SSP-DOX. (a) TEA in CH_2Cl_2, 0 oC, then methacryloyl chloride in CH_2Cl_2 added, rt, 12 h; (b) 50 % TFA in CH_2Cl_2, rt. 1 h, then DOX•HCl, catalytic amount TFA, in anhydrous CH_3OH, 48 h in dark; (c) TEA and (3-mercaptopropyl) trimethoxysilane in anhydrous DMF, rt, 12 h.

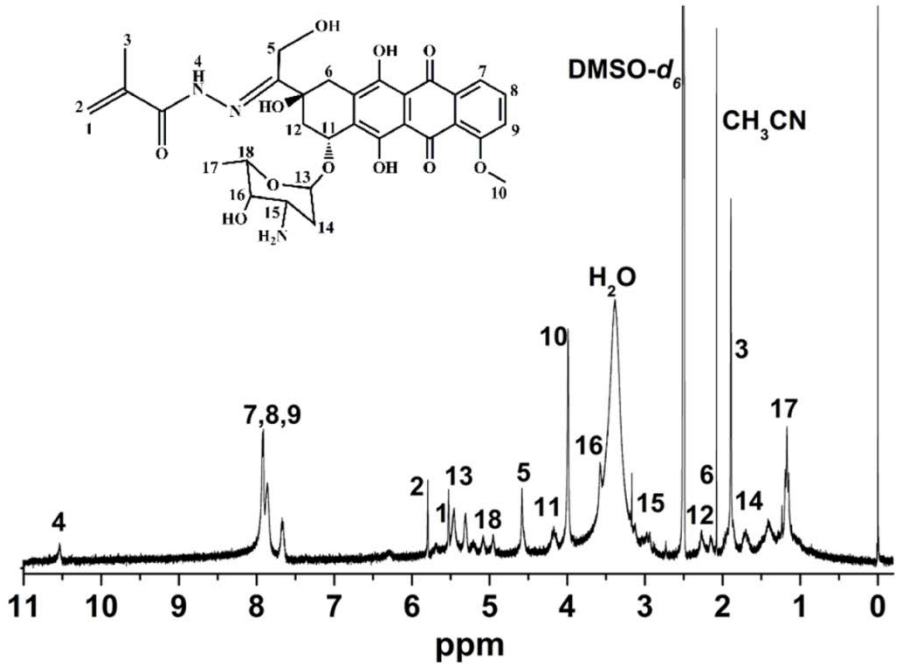

Figure S5. ^{1}H NMR spectrum (300 MHz, Dimethyl sulfoxide-d_6) of BH-DOX.

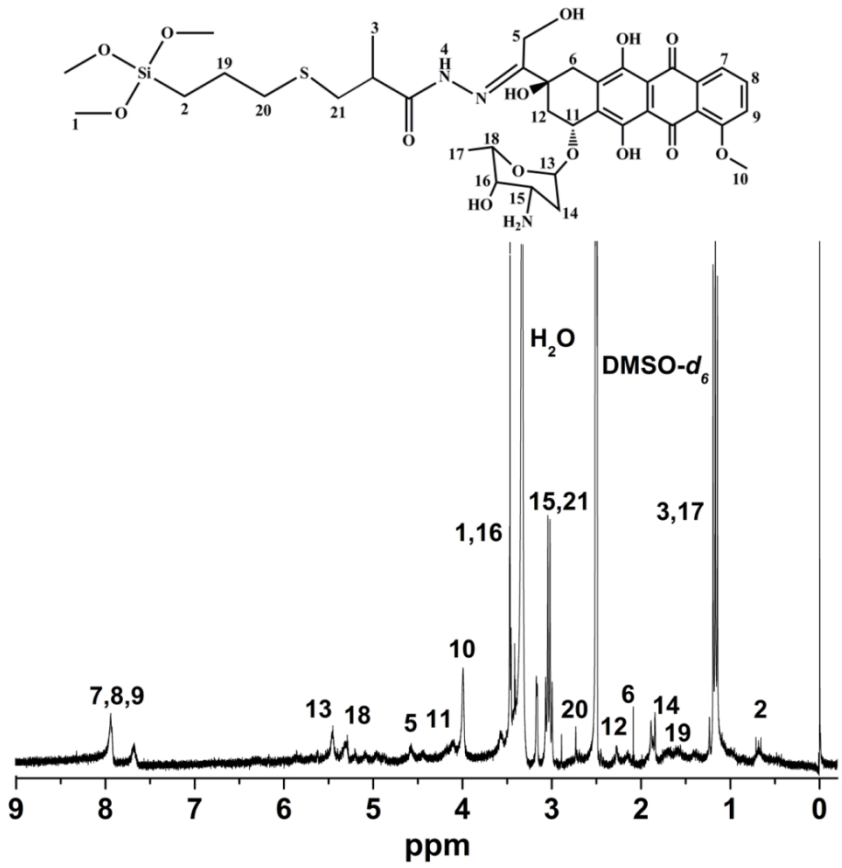

Figure S6. ^{1}H NMR spectrum (300 MHz, Dimethyl sulfoxide-d_6) of SSP-DOX.

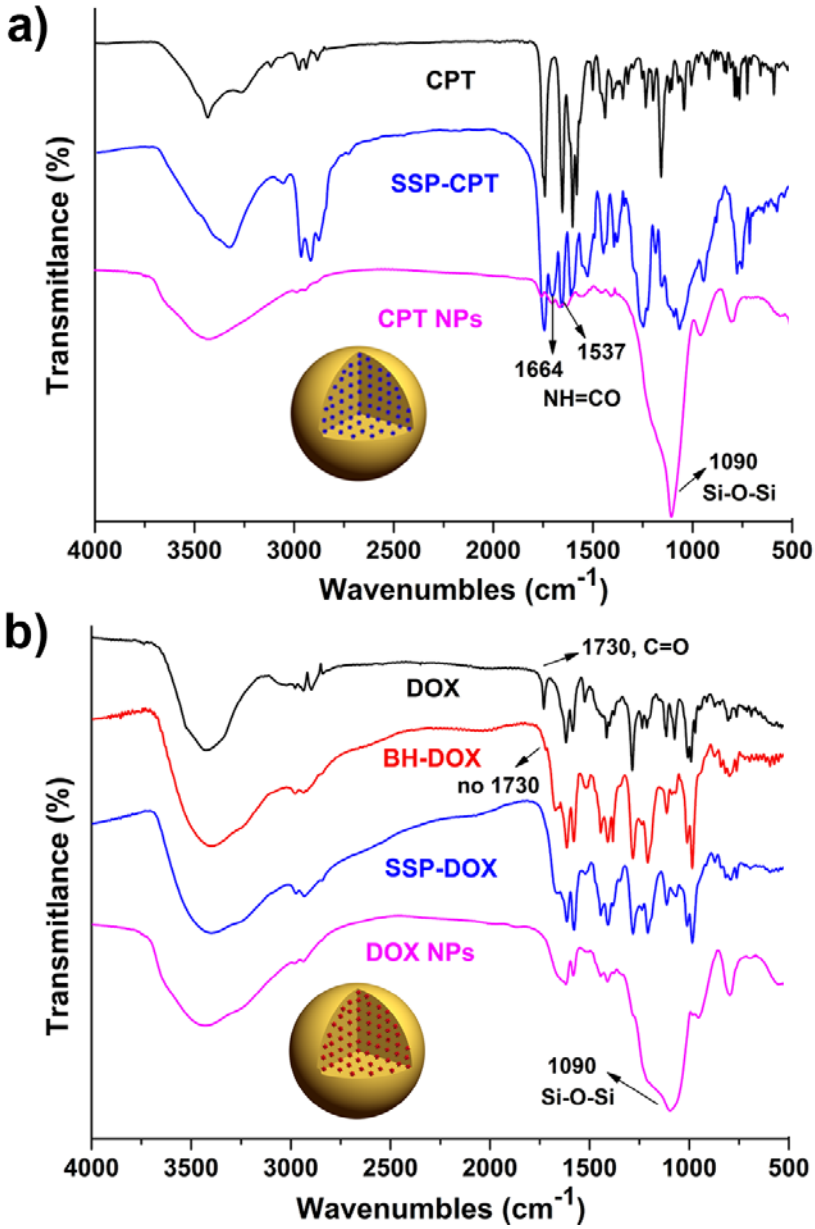

Figure S7. FTIR spectra of CPT, SSP-CPT monomer and CPT NPs (a); DOX, BH-DOX, SSP-DOX monomer and DOX NPs (b).

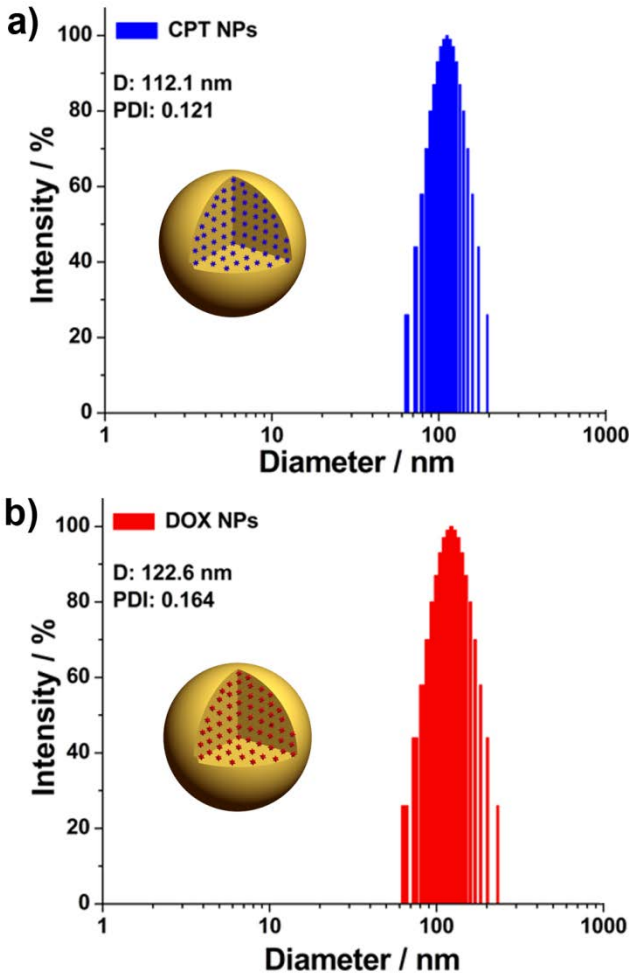

Figure S8. Size distribution of CPT NPs (a) and the DOX NPs (b) determined by dynamic laser light scattering (DLS).

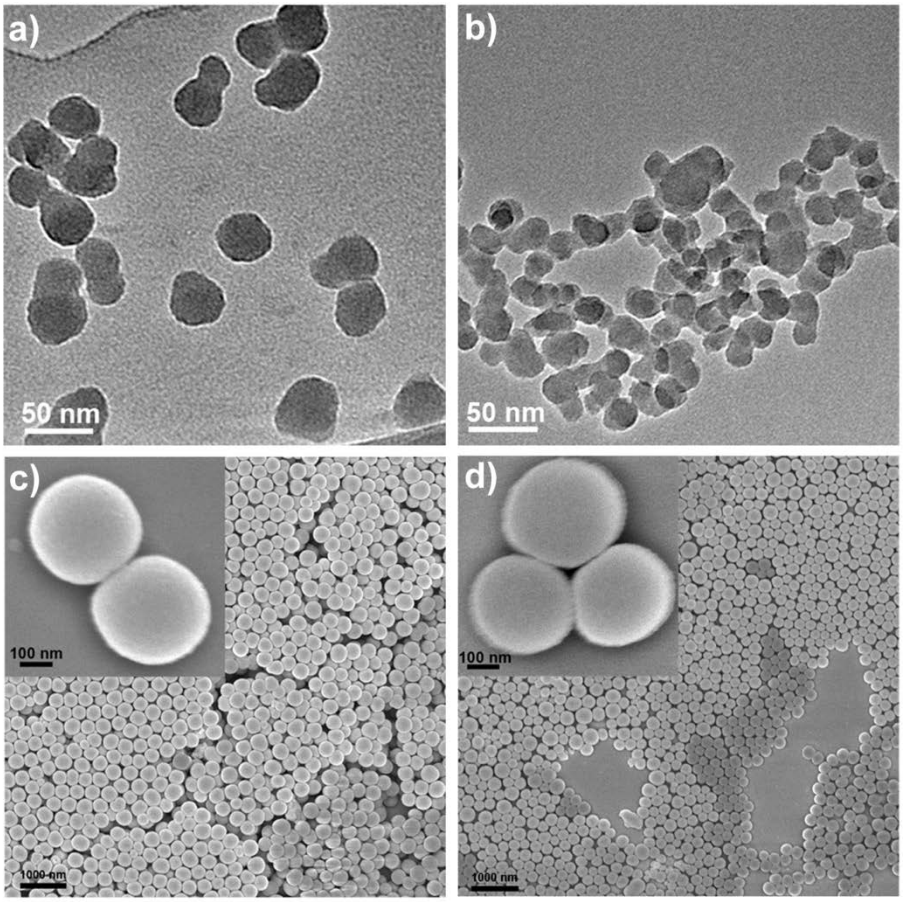

Figure S9. (a-b) TEM images of CPT-50 NPs (a) and DOX-50 NPs (b) with an average diameter of 50 nm. (c-d) SEM images of CPT-200 NPs (c) and the DOX-200 NPs (d) with an average diameter of 200 nm.

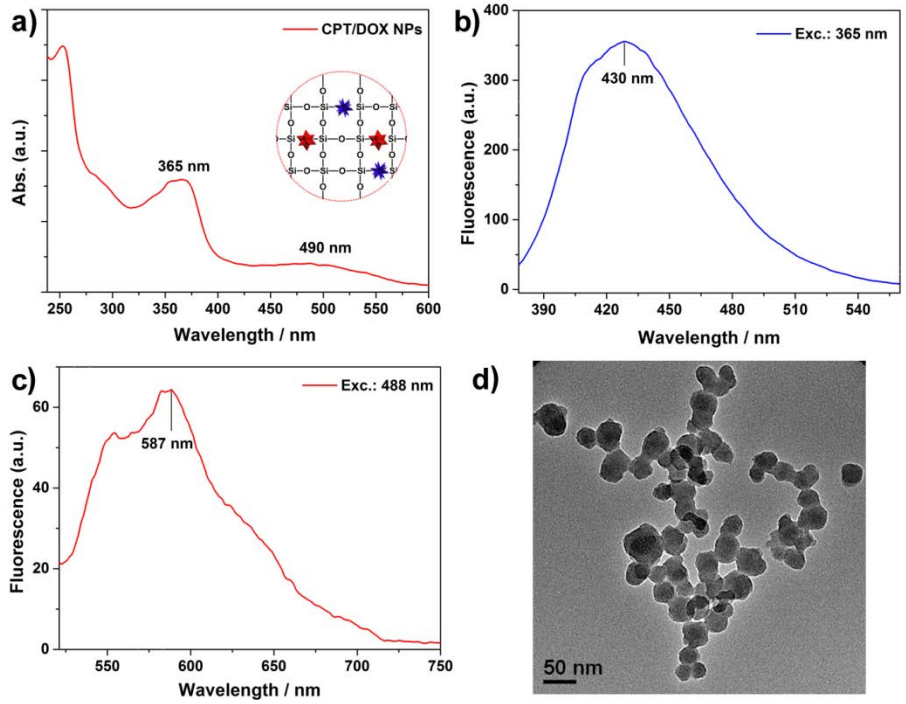

Figure S10. (a) UV-vis absorption spectrun of CPT/DOX NPs; (b) The photoluminescence spectra of CPT/DOX NPs with different excitation wavelength of (b) 365 nm and (c) 488 nm; (d) TEM image of CPT/DOX NPs-100.

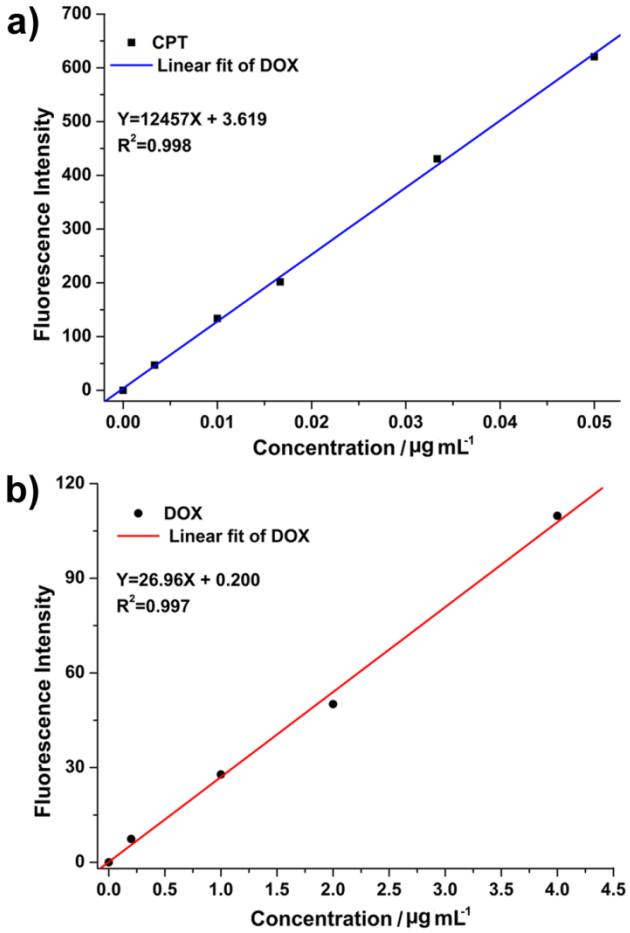

Figure S11. (a) The fluorescence intensity of CPT at emission wavelength of 440 nm, excitation wavelength of 365 nm, and slit width of 5 nm as a function of CPT concentration. (b) The fluorescence intensity of Dox at emission wavelength of 560 nm, excitation wavelength of 488 nm, and slit width of 5 nm as a function of Dox concentration.

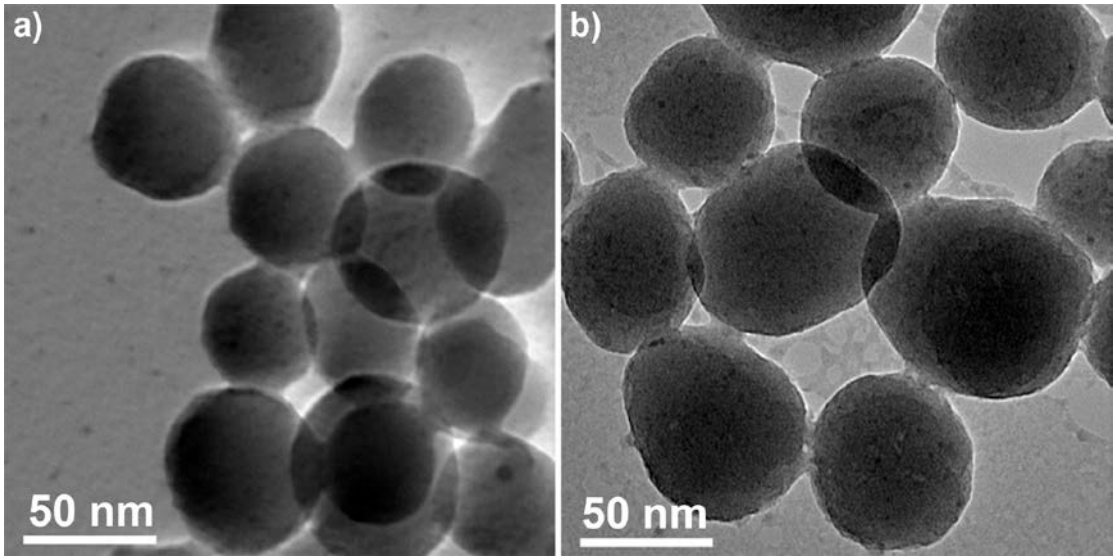

Figure S12. TEM images of the freshly prepared CPT NPs (a) and DOX NPs (b) after being incubated with PBS (50 mM, pH 7.4) for 120 h, respectively.

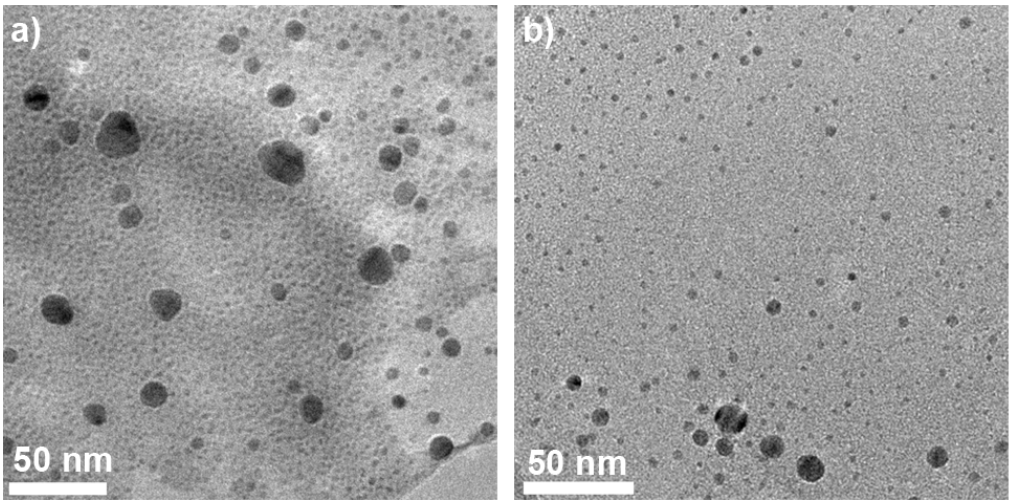

Figure S13. TEM image of (a) CPT NPs after being incubated in PBS (50 mM, pH 7.4) containing 10 mM GSH for 120 h and (b) DOX NPs after being incubated in PBS (50 mM, pH 5.0) for 120 h, respectively.

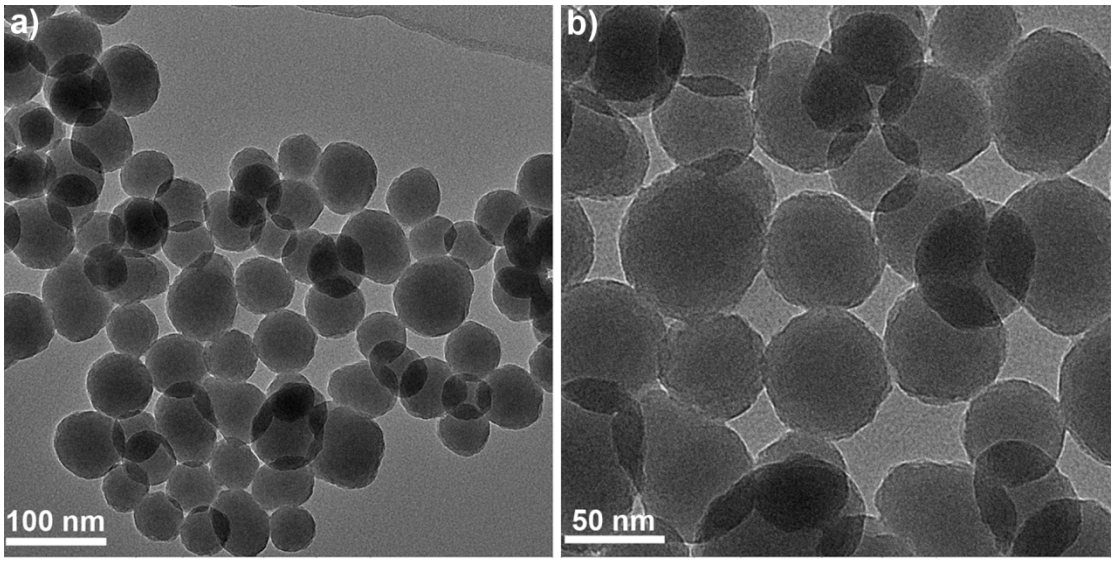

Figure S14. TEM images of the freshly prepared SiO_2 NPs-100 (a and b) after being incubated with PBS (50 mM, pH 7.4) containing 10 mM GSH for 72 h.

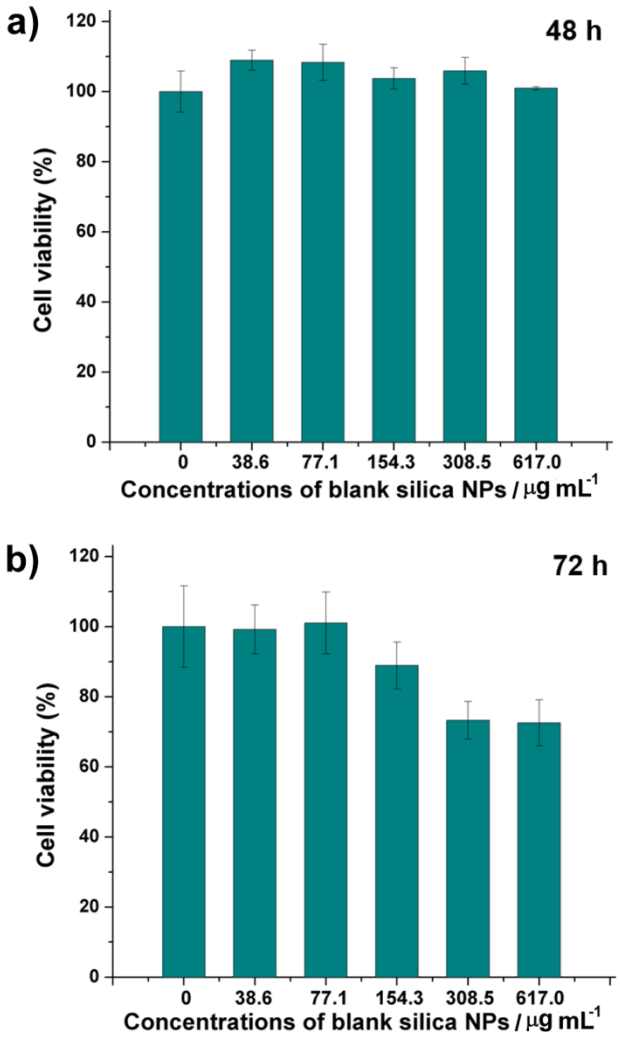

Figure S15. Relative cell viability of HeLa cells after treatment with different concentrations of blank silica NPs-100 for 48 h (a) and 72 h (b) tested by PrestoBlue assay. Cells without treatment were used as control. Data were shown as means ± SD (n=3).

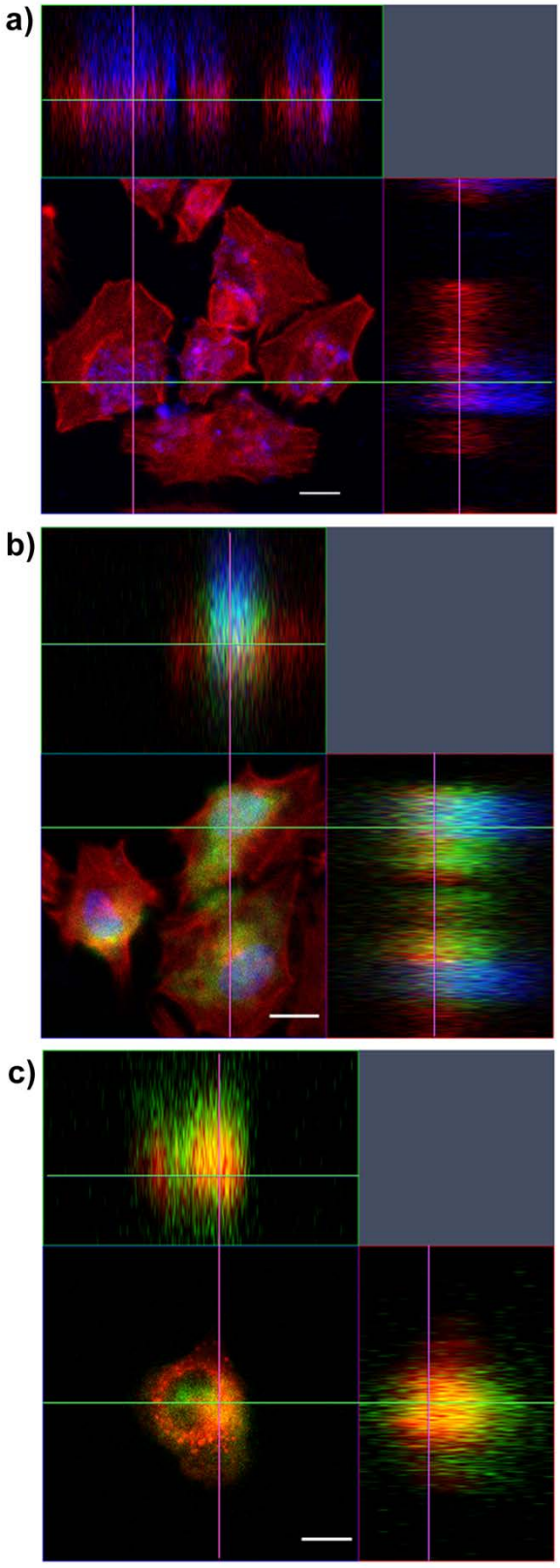

Figure S16. Corresponding confocal laser scanning microscopy ortho images in Figure 4 of HeLa cells showing different z-stack.

Table S1: Reaction Conditions of Size-Controlled CPT or DOX Silica Prodrug NPs.[a]

Sample	MeOH (mL)	Water (μL)	NH_4OH (μL)	TEOS (mg)	SSP Drug (mg)
CPT NPs-50	1.0	360	85	60	2.0
CPT NPs-100	1.0	360	110	60	2.0
CPT NPs-200	1.0	270	240	60	2.0
DOX NPs-50	1.0	360	85	60	2.0
DOX NP_S-100	1.0	360	110	60	2.0
DOX NP_S-200	1.0	270	240	60	2.0

[a)] Stirring rate and the reaction time of all samples are 100 rpm and 12 h, respectively.

Table S2: The structure information of Size-Controlled CPT NPs or DOX NPs.[a]

Sample	Size[a] (nm)	PDI[a]	ζ-potential	LC (wt %)
CPT NPs-50	47.1	0.238	-17.89	4.5
CPT NPs-100	112.1	0.121	-16.09	3.6
CPT NPs-200	232.5	0.091	-16.81	2.3
DOX NPs-50	52.9	0.293	-22.03	4.3
DOX NP_S-100	121.8	0.244	-24.59	3.3
DOX NP_S-200	251.6	0.005	-26.69	2.8

[a)] The size and PDI of resulting prodrug NPs were determined by DLS.

Table S3: IC_{50} value of free drug and prodrug NPs-100 of different time.[a]

Sample	IC_{50} (24 h) μg mL^{-1}	IC_{50} (48 h) μg mL^{-1}	IC_{50} (72 h) μg mL^{-1}
Free CPT	9.0	0.52	0.48
Free DOX	1.2	0.43	0.62
[b]Free CPT/ Free DOX	0.51	0.33	0.51
CPT NPs	> 10	3.8	0.63
DOX NPs	> 10	4.7	0.61
[c]CPT NPs/DOX NPs	0.56	1.16	0.52

[a)] IC_{50} value of free CPT, free DOX, CPT NPs and DOX NPs determined in HeLa cells by PrestoBlue assay, and cells were incubated with samples for different time.
[b,c)] The total drug concentrations ranging from 0.625-10 μg equiv/mL for free drug and prodrug NPs, and the mass ratio is 1:1.