Supporting information

Novel multi-stimuli responsive functionalized PEG-based co-delivery nanovehicles toward sustainable treatments of multidrug resistant tumor

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Materials and reagents

Ferrocenecarboxylic acid (FCA, 98%), hydroxyethyl methacrylate, (HEMA, 99%), 4-dimethylaminopyridine (DMAP, 99%), N, N'-dicyclohexylcarbodiimide (DCC, 99%), selenium powder (Se, 99.99%), 3-chloropropionic acid (CPA, 98%) and paclitaxel (PTX, 98%) were purchased from Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Sodium borohydride ($NaBH_4$, 96%), anhydrous magnesium sulfate ($MgSO_4$,⩾99.0%), anhydrous sodium carbonate (Na_2CO_3, ⩾99.8%), dichloromethane (DCM) were supplied by Sinopharm Chmical Reagent Co. Ltd. (Shanghai, China).

Synthesis of monomer MAOEFC

FCA (4.6 g, 20 mmol) and HEMA (3.4 mL, 28 mmol) were dissolved in anhydrous DCM (250 mL) completely, then DMAP (2.44 g, 20 mmol) was also blended into the above solution. The obtained solution was shifted in ice bath under nitrogen atmosphere with stirring for 3 h. DCC (5 g, 24 mmol) was dissolved in DCM (30 mL) afterwards, and dropwise added into the mixture within 30 h through constant pressure funnel. The esterification was carried out at 25^{o}C for 24 h. Insoluble DCU was removed by filtration, and then rotary evaporation was performed to remove the excess DCM. The mixture was extracted thrice using saturate sodium bicarbonate solution and deionzied water to remove DMAP and unreacted FCA. The liquor was separated by passing through silica gel column chromatography and employing petroleum ether and ethyl acetate (*v*/*v*=10/1) as eluent. The orange solid MAOEFC was obtained through rotary evaporation and follow-up vacuum drying at room temperature. (*Yield*: 57%) (^{1}H NMR, 300 MHz, $CDCl_3$), δ (ppm): 1.99 (t, 3H), 6.29

(dd, 1H), 5.56 (dd, 1H), 4.92 (s, 2H, *meta*-H in $–C_5H_4$), 4.55 (m, 4H, -$OCH_2\underline{CH_2}$O-), 4.51 (s, 2H, *ortho*-H in C_5H_4), 4.29 (s, 5H, C_5H_5). FT-IR (KBr pellets) ν (cm^{-1}) 3030-3107 (=C-H), 2875-2985 (-C-H stretching vibration), 1711 (-C=O), 1610 (-C=C-), 1180-1220 (-C-O-), 770-855 (=C-H in C_p), 434-510 (Fe-C/C_p-Fe).

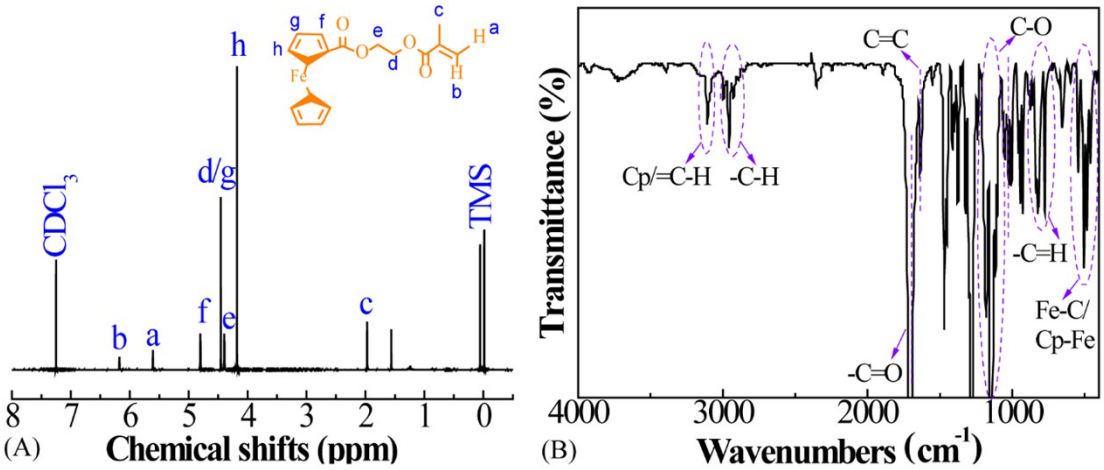

Figure S1 Synthetic scheme (A), ^{1}H NMR (B) and FTIR (C) spectra of monomer MAOEFC.

Synthesis of 3, 3'-diselenodipropionic acid

The detailed synthetic procedure of 3,3'-diselenodipropionic acid **(**DSeDPA) has been recorded in our previous work. [1] Selenium powder (2.37 g, 30 mmol) was mixed with deionized water (10 mL) under nitrogen atmosphere, $NaBH_4$ (2.27 g, 60 mmol) was dissolved in deionized water (25 mL) and added into the above solution with stirring in ice bath till the mixture became colorless afterwards. The bottle was heated at 105 ^oC for 20 min, and selenium powder (2.37 g, 30 mmol) was blended and the liquor became reddish. CPA (6.50 g, 60 mmol) was dissolved in deionized water (15 mL) and Na_2CO_3 was used to adjust pH of the solution till 8.0, after that, the mixture was injected into the system. The reaction was performed at 25^oC for 16 h. Then the mixture was exposed in air and precipitated static settled. The liquor needed to be filtrated and adjusted the pH till 3~4 using HCl (1M), followed by extracted with ethyl acetate thrice, organic phase was collected and dried with anhydrous $MgSO_4$ overnight, the crude product was obtained through rotary evaporation and further purified in recrystallization method with ethyl acetate. The yellow solid DSeDPA was vacuum dried at 40 ^oC. (*Yield*: 81.3%) (^{1}H NMR, 300 MHz, DMSO-*d6*), δ (ppm): 2.71 (t, 2H, -SeCH_2CH_2-), 3.05 (t, 2H, -Se CH_2CH_2-), 12.38 (s, 1H, -Se CH_2CH_2COOH). FTIR (KBr pellets): ν (cm^{-1}) 3460 (-OH stretch in -COOH), 1700 (carboxylic –C=O stretch), 1238 (-C-O stretch), 527-706 (C-Se) and 2898-3030 (-C-H stretch).

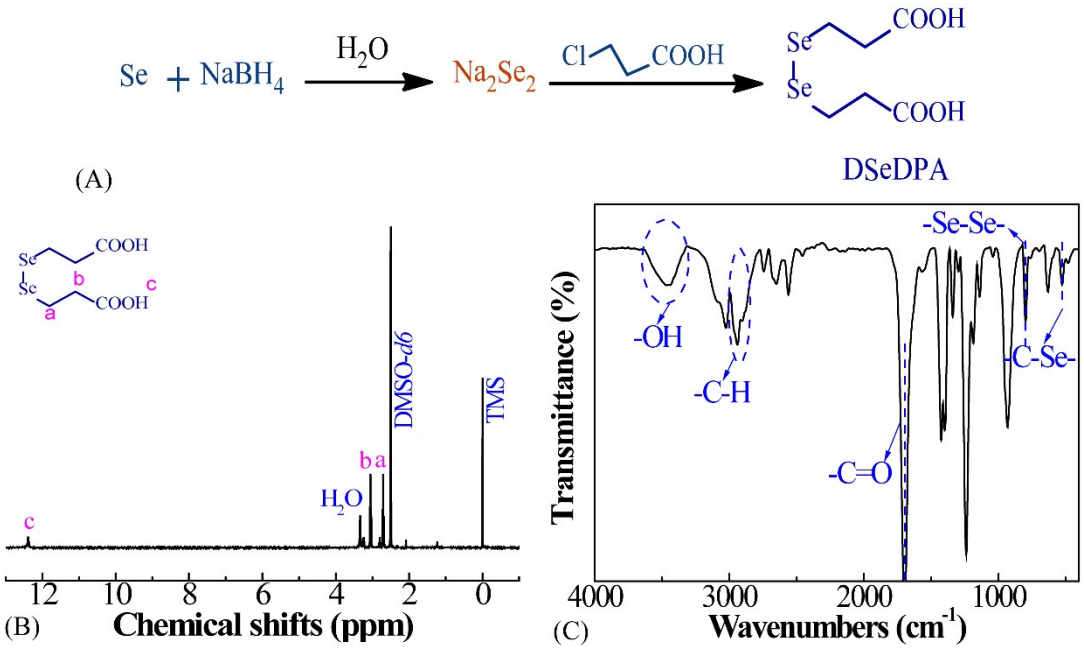

Figure S2 Synthetic scheme (A), ^{1}H NMR (B) and FTIR (C) spectrums of DSeDPA.

Synthesis of diselenium linked PTX dimer PTX-SeSe-PTX

PTX dimer was prepared using the DSeDPA containing diselenium bond as linker to generate PTX-SeSe-PTX. Paclitaxel (PTX) (85 mg, 0.1 mmol) was dissolved in anhydrous DCM (10 mL). DSeDPA (15.2 mg, 0.05 mmol), EDC·HCl (38 mg, 0.2 mmol) and DMAP (1.2 mg, 0.01 mmol) were added successively with stirring at room temperature for 1 h. Afterwards, EDC·HCl (19 mg, 0.1 mmol) and DMAP (1.2 mg, 0.01 mmol) were added the aforementioned system and the reaction was carried out overnight. The mixture was quenched using saturated ammonium chloride solution, and washed with distilled water and salt solution. The liquor was dried with anhydrous $MgSO_4$, followed by filtration, rotary evaporation. The concentrated solution was separated by passing silica gel column chromatography with ethyl acetate and hexane (*v*/*v*=1:1) as eluent. (*Yield*: 35%)

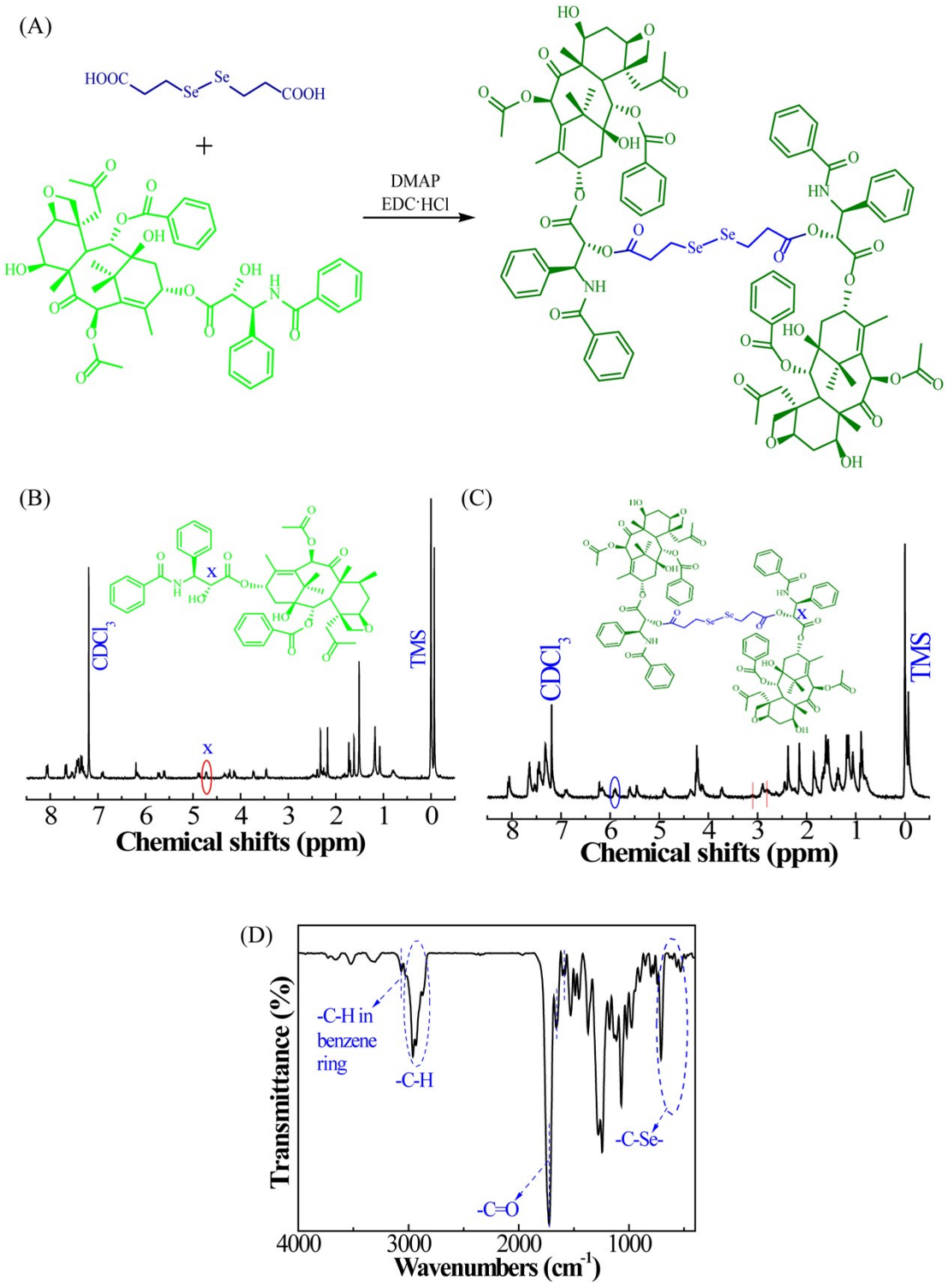

Figure S3 Synthetic scheme (A) and ^{1}H NMR spectrum of (B) PTX, (C) PTX-SeSe-PTX. FTIR spectrum (D) of PTX-SeSe-PTX.

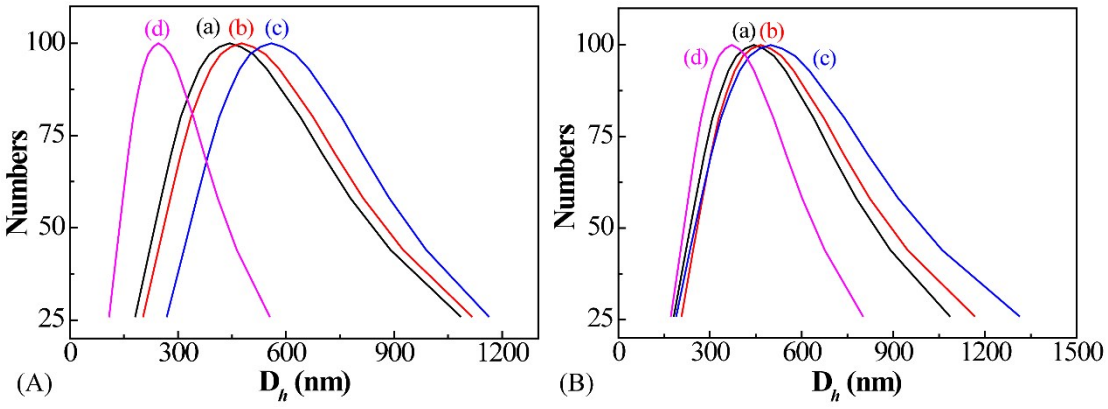

Figure S4 D_h changes of P-DOX$^{@PTX\ dimer}$ micelles reduced by 10 mM GSH (A) and oxidized by 100 μM H_2O_2 (B) for 0 (a), 2 (b), 6 (c) and 48 h (d).

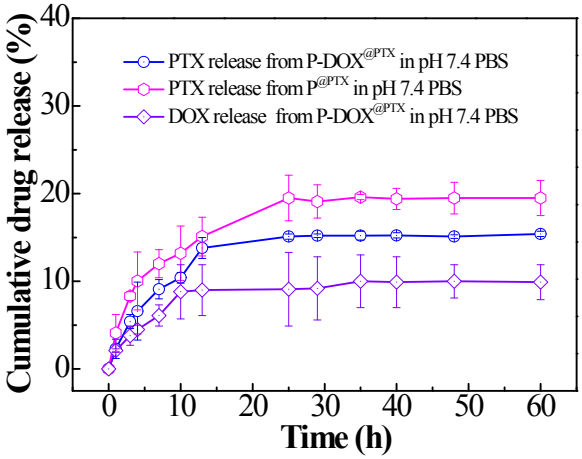

Figure S5. DOX and PTX release profiles from P-DOX$^{@PTX}$ and P$^{@PTX}$ in pH 7.4 PBS.

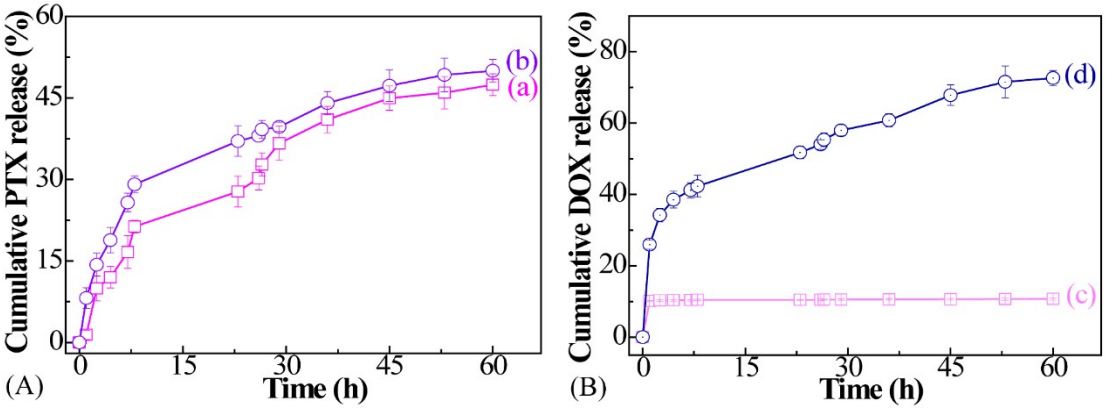

Figure S6 PTX (A) and DOX (B) release profiles from P-DOX$^{@PTX\ dimer}$ micelles in pH 7.4 PBS with 0.5% NaClO (a, c) and pH 5.0 PBS with 0.5% NaClO (b, d) for 60 h.

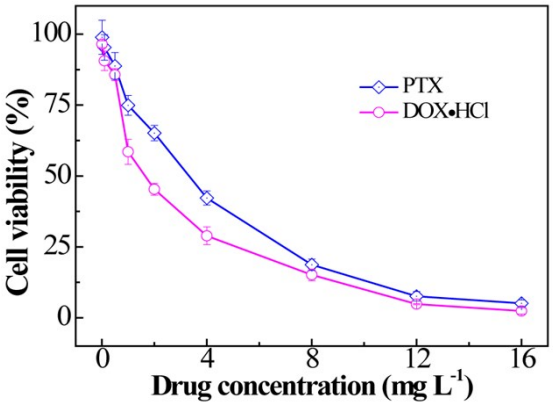

Figure S7 Cytotoxicity of free drugs against Hela cells (n=3) after 48 h.

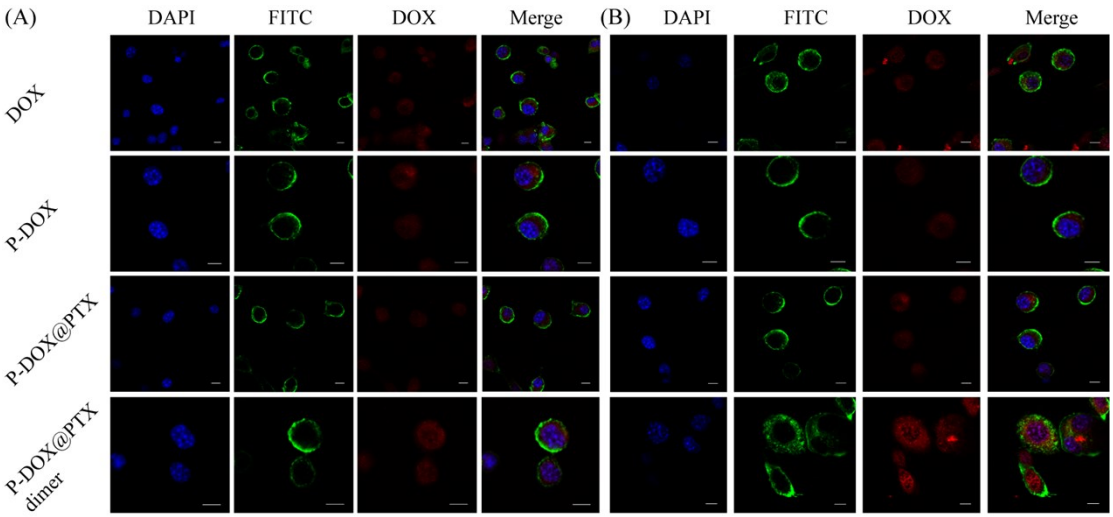

Figure S8 Qualitative analysis of cell uptake of DOX by CLSM in HCT116 (A) and HCT116/ADR (B) cells after 1 h incubation

Reference

[1] Xu, J.-W.; Ge, X.; Lv, L.-H.; Xu, F.; Luo, Y.-L. Dual-stimuli-responsive paclitaxel delivery nanosystems from chemically conjugate self-assemblies for carcinoma treatment. *Macromol. Rapid Commun.* **2018**, *39*, 1800628.