Electronic Supplementary Information

Glutathione-depleting polymer delivering chlorin e6 for enhancing photodynamic therapy

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Synthesis methods

Synthesis of $mPEG_{2k}$-DDAT

$mPEG_{2k}$-DDAT was synthesized by the esterification reaction between terminal hydroxyl group in $mPEG_{2k}$-OH and carboxyl group in DDAT. Firstly, 2-Methyl-2-[(dodecylsulfanylthiocarbonyl) sulfanyl] propanoic acid (DDAT) was synthesized. 2-Mercaptoethanol (8.1 g), 18-corone-6 ether (0.5 g) and acetone (20 g) were mixed under the protection of N_2. The reaction was kept at 0 ℃ in an ice bath. Then 50% mass fraction of NaOH solution (1.7 g) was slowly dropped into the reaction flask. After 30 min stirring, carbon disulfide (3.2 g) dissolved in 10mL acetone, trichloromethane (7.5 g) and 50% mass fraction of NaOH solution (8 g) were slowly dropped into the reaction system, respectively. Then the reaction was stirred at room temperature overnight. Deionized water (60 mL) was added, and the system acidified by hydrochloric acid until pH=2. Rotary evaporation was used to remove acetone of the mixture. After that, the mixture was separated by filtration. The filtrate residue was redissolved in 50 mL isopropyl alcohol, and the solution was filtered again to obtain filtrate. Recrystallization was carried out twice in n-hexane, and DDAT was obtained as golden yellow needle-like crystal. Secondly, $mPEG_{2k}$-OH (1 g, 0.5 mmol, 1 eq), DDAT (0.182 g, 5 mmol, 10 eq), EDCI (0.479 g, 2.5 mmol, 5 eq), DMAP (0.305 g, 2.5 mmol, 5 eq) and a small amount of anhydrous $MgSO_4$ were added into a 50 mL Schlenk flask under an N_2 atmosphere. The mixture was dissolved in dry dichloromethane and the reaction was stirred at room temperature for 48 h. After that, the mixture was filtered. The filtrate was precipitated in diethyl ether, and washed with diethyl ether for three times. Finally, $mPEG_{2k}$-DDAT was successfully synthesized. The NMR spectrum was showed in **Figure S2**.

^{1}H NMR (400 MHz, Chloroform-*d*), δ (ppm): 3.62–3.73 (m, 178H, -O-(C***H***$_2$)$_2$-O-), 3.39 (s, 3H, C***H***$_3$-O-(CH$_2$)$_2$-O-), 1.71 (s, 6H, -CO-C(C***H***$_3$)$_2$-S-), 1.25-1.47 (m, 22H, -S-(C***H***$_2$)$_{11}$-CH$_3$), 0.89 (t, J = 6.8 Hz, 3H, -S-(CH$_2$)$_{11}$-C***H***$_3$).

Synthesis of hydroxyethylpyridyldisulfide (HPDS)

2, 2'-dipyridyl disulfide (DPDS) (10.3 g, 0.047 mol) dissolved in 80 mL methanol and catalyst glacial acetic acid (1.2 mL) were added into a 250 mL round bottom flask. Mercaptoethanol (4.4 g, 0.056 mol, 4 mL) in 50 mL methanol was dropped into the mixture. Then the reaction was kept at room temperature overnight. After that, solvent methanol was removed by evaporation. The crude product (yellow oil) was separated and purified by flash column chromatography (silica gel as stationary phase and 40% of ethyl acetate/hexane as eluent).

Synthesis of PyDSMA and PyEMA

12 g Hydroxyethylpyridyldisulfide (HPDS) or 7.9 g 2-(2-hydroxyethyl) pyridine (DP) dissolved in 50 mL dry DCM and triethylamine (7.8 g, 76.9 mmol) were mixed and kept at 0 ℃ in an ice bath. Methacryloyl chloride (6.7 g, 64.1 mmol) dissolved in 30 mL dry DCM was dropped into the mixture. Then the reaction was stirred at room temperature overnight. After that, the reaction

product was firstly washed with 100 mL deionized water for three times and then washed with 100 mL brine. The organic layer was dried with anhydrous Na_2SO_4 and concentrated to obtain the yellow oil by evaporation. The crude product (yellow oil) was separated and purified by flash column chromatography (silica gel as stationary phase and 30% of ethyl acetate/hexane as eluent). Finally the monomer PyDSMA or PyEMA was successfully synthesized. The NMR spectra was showed in **Figure S3** and **S4**.

^{1}H NMR of PyDSMA: (400 MHz, Chloroform-*d*), δ (ppm): 8.49 (m, 1H, aromatic proton *ortho*-N), 7.72 (m, 1H, aromatic proton *meta*-N), 7.65 (m, 1H, aromatic proton *para*-N), 7.12 (m, 1H, aromatic proton *ortho*-disulfide linkage), 6.15 (d, J = 1.3 Hz, 1H, vinylic proton, *cis*-ester), 5.61 (d, J = 1.7 Hz, 1H, vinylic proton, *trans*-ester), 4.42 (t, J = 6.4 Hz, 2H, -S-S-$CH_2C\underline{\boldsymbol{H}}_2O$-), 3.12 (t, J = 6.4 Hz, 2H, -S-S-$C\underline{\boldsymbol{H}}_2CH_2O$-), 1.96 (s, 3H, methyl proton of the methacryloyl group).

^{1}H NMR of PyEMA: 400 MHz, Chloroform-*d*), δ (ppm): 8.56 (m, 1H, aromatic proton *ortho*-N), 7.62 (m, 1H, aromatic proton *para*-N), 7.18 (m, 2H, aromatic proton *meta*-N and *ortho*-disulfide linkage), 6.04 (d, J = 2.0, 1.0 Hz, 1H, vinylic proton, *cis*-ester), 5.53 (d, J = 1.6 Hz, 1H, vinylic proton, *trans*-ester), 4.54 (t, J = 6.7 Hz, 2H, Py-$CH_2C\underline{\boldsymbol{H}}_2O$-), 3.17 (t, J = 6.7 Hz, 2H, Py-$C\underline{\boldsymbol{H}}_2CH_2O$-), 1.90 (s, 3H, methyl proton of the methacryloyl group).

Synthesis of PEG-p(PyDSMA) and PEG-p(PyEMA)

PEG-p(PyDSMA) and PEG-p(PyEMA) polymers were synthesized by Reversible Addition-Fragmentation Chain Transfer Polymerization (RAFT). Briefly, the monomer PyDSMA or PyEMA (3.06 g, 12 mmol or 2.29 g, 12 mmol), macromolecular chain transfer agent $mPEG_{2k}$-DDAT (0.8 g, 0.4 mmol) and initiator AIBN (20 mg) were dissolved in 8 mL dioxane under an N_2 atmosphere. The mixture was bubbled with N_2 for another 30 min to completely remove oxygen. Then the reaction was kept at 70 °C for 20 h. After that, the reaction was stopped by rapidly cooling it down. 15 mL DMF was added to dilute the reaction solution. The mixture was dialyzed (MW cut-off: 1000 Da) against deionized water and freeze-dried. Finally, the polymer PEG-p(PyDSMA) or PEG-p(PyEMA) was successfully synthesized. The NMR spectra was showed in **Figure 2A**.

^{1}H NMR of PEG-p(PyDSMA): (400 MHz, Chloroform-*d*), δ (ppm): 8.46 (m, 1H, aromatic proton *ortho*-N), 7.68 (m, 2H, aromatic proton *meta*-N and *para*-N), 7.10 (m, 1H, aromatic proton *ortho*-disulfide linkage), 4.23 (t, 2H, -S-S-$CH_2C\underline{\boldsymbol{H}}_2O$-), 3.62–3.73 (m, 178H, -O-($C\underline{\boldsymbol{H}}_2$)$_2$-O-), 3.39 (s, 3H, $C\underline{\boldsymbol{H}}_3$-O-($CH_2$)$_2$-O-), 3.03 (t, 2H, -S-S-$C\underline{\boldsymbol{H}}_2CH_2O$-), 1.94 (m, 2H, -($C\underline{\boldsymbol{H}}_2$-C($CH_3$))$_n$-), 0.80 (m, 3H, -($CH_2$-C($C\underline{\boldsymbol{H}}_3$))$_n$-).

^{1}H NMR of PEG-p(PyEMA): (400 MHz, Chloroform-*d*), δ (ppm): 8.54 (m, 1H, aromatic proton *ortho*-N), 7.63 (m, 1H, aromatic proton *para*-N), 7.18 (m, 2H, aromatic proton *meta*-N and *ortho*-disulfide linkage), 4.33 (t, J = 7.3 Hz, 2H, Py-$CH_2C\underline{\boldsymbol{H}}_2O$-), 3.62–3.73 (m, 178H, -O-($C\underline{\boldsymbol{H}}_2$)$_2$-O-), 3.39 (s, 3H, $C\underline{\boldsymbol{H}}_3$-O-($CH_2$)$_2$-O-), 3. 10 (t, J = 6.6 Hz, 2H, Py-$C\underline{\boldsymbol{H}}_2CH_2O$-), 1.69 (m, 2H, -($C\underline{\boldsymbol{H}}_2$-C($CH_3$))$_n$-), 0.78 (m, 3H, -($CH_2$-C($C\underline{\boldsymbol{H}}_3$))$_n$-).

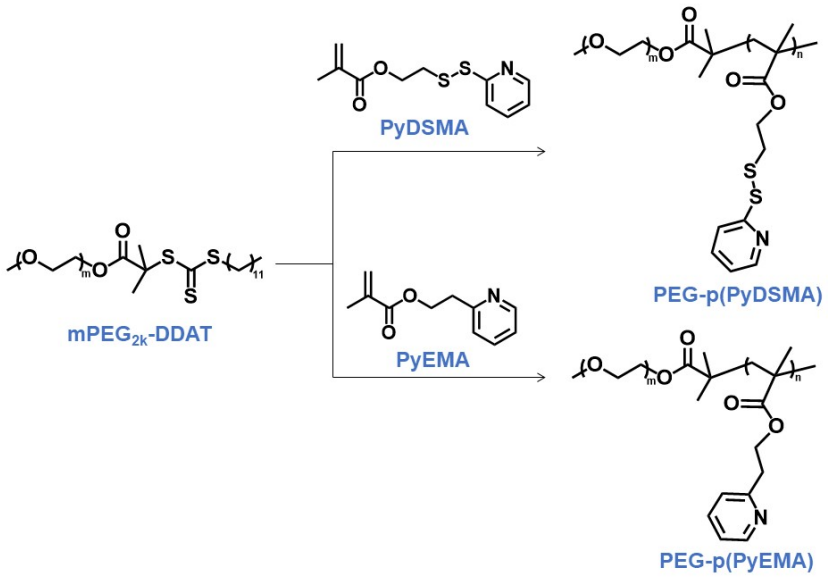

Fig. S1 The synthetic route of GSH-depleting polymer PEG-p(PyDSMA) and GSH-nondepleting polymer PEG-p(PyEMA)

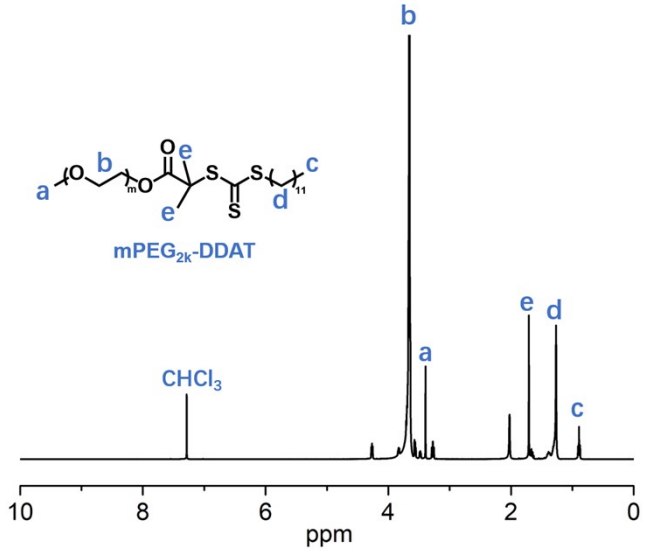

Fig. S2 ^{1}H-NMR spectra of mPEG$_{2k}$-DDAT in $CDCl_3$

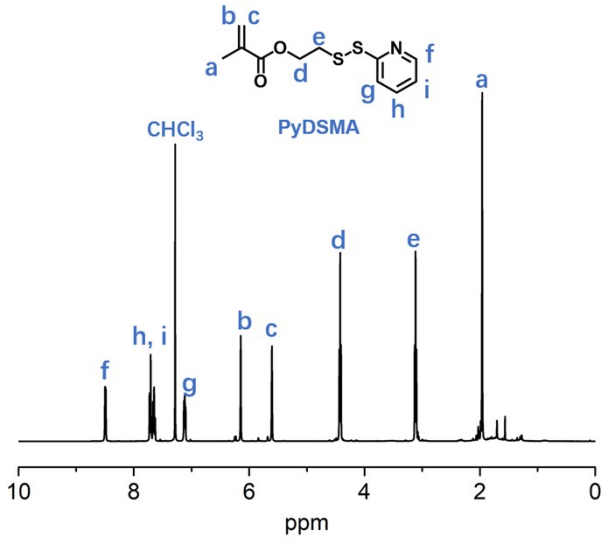

Fig. S3 ^{1}H-NMR spectra of PyDSMA in $CDCl_3$

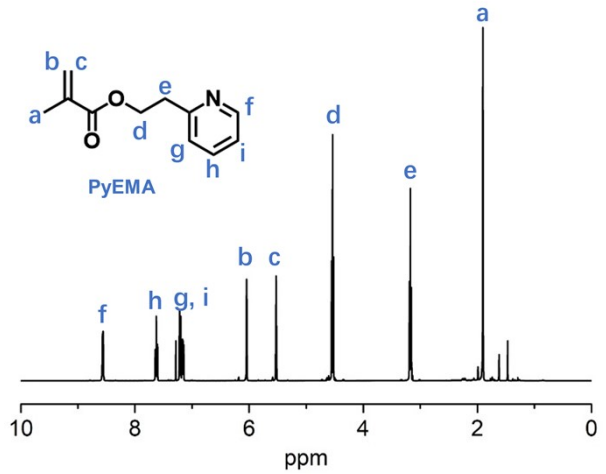

Fig. S4 ^{1}H-NMR spectra of PyEMA in $CDCl_3$

Polymer	M_n	PDI
PEG-p(PyDSMA)	8600	1.28
PEG-p(PyEMA)	7900	1.22

Fig. S5 The molecular weights (M_n) and polydispersity index (PDI) of PEG-p(PyDSMA) and PEG-p(PyEMA) polymers detected by GPC analysis.

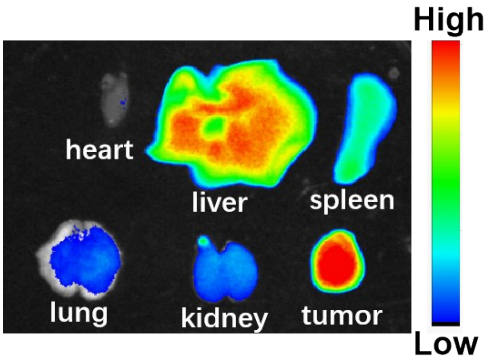

Fig. S6 The fluorescence of heart, liver, spleen, lung, kidneys and tumor after dissection.

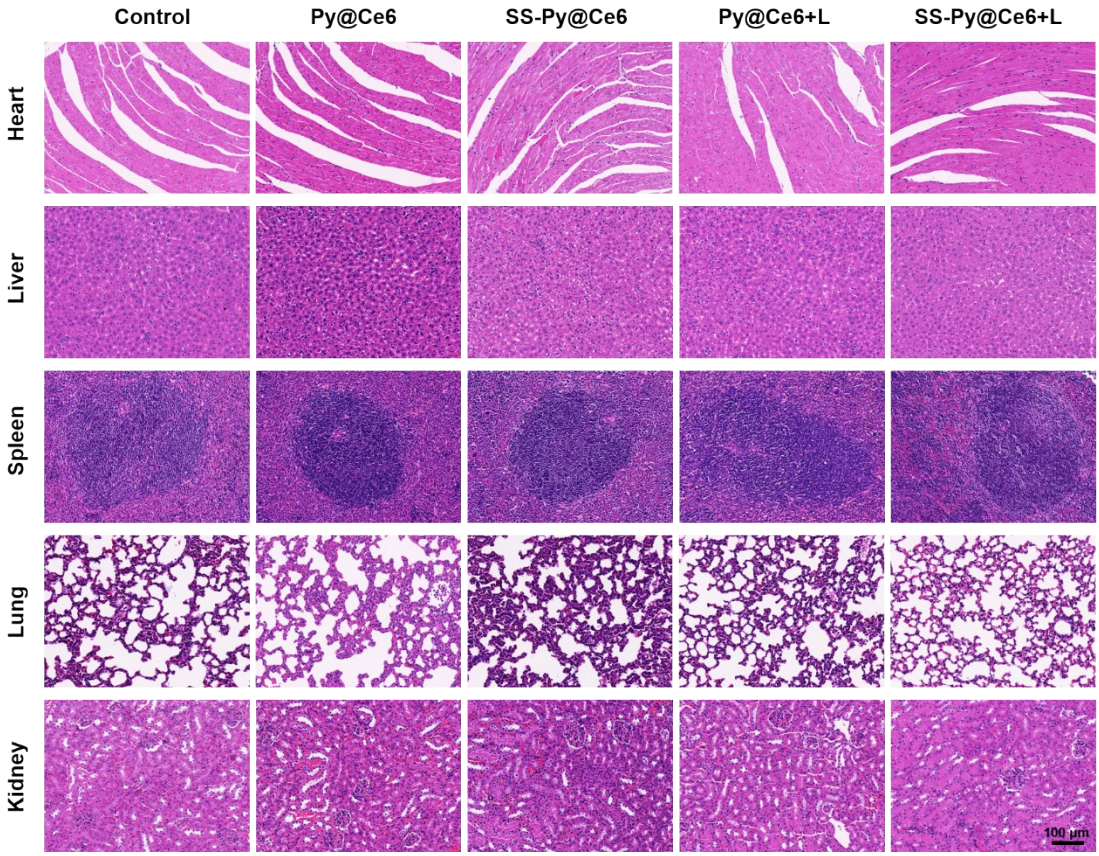

Fig. S7 The H&E staining of excised major organ tissues of mice (heart, liver, spleen, lung, kidneys). Scale bar, 100 μm.