Supporting information of

Leveraging a polycationic polymer to direct tunable loading of anticancer agent and photosensitizer with opposite charges for chemo-photodynamic therapy

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1. Materials

2,2-Bis(hydroxymethyl)propionic acid (BHPA) was purchased from Heowns (China, Tianjin). Allyl bromide and 2,2-dimethoxy-2-phenylacetopheone (DMPA) were supplied from Meryer (Shanghai) Chemical Technology Co., Ltd. Methoxy poly(ethylene glycol) (mPEG₂₀₀₀), stannous octoate (Sn(Oct)₂), and cysteamine hydrochloride were purchased from Sigma-Aldrich. Doxorubicin hydrochloride (DOX·HCl) was obtained from Yingxuan Chempharm Co. (Shanghai, China). Chlorin e6 (Ce6) was purchased from J&K Chemical LTD. (China, Shanghai). Potassium hydroxide and organic solvents were provided by Kelong Chemical Co., Ltd. (Chengdu, China) Triethylamine (TEA) was purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. Singlet oxygen sensor green (SOSG) was purchased from Dalian Meilun Biotechnology Co.,Ltd. All the chemicals were used as obtained unless otherwise mentioned.

Dulbecco's Modified Eagle's Medium (DMEM) and Roswell Park Memorial Institute (RPMI) 1640 medium were supplied from Shanghai Basal Media Technologies Co., LTD. Fetal bovine serum (FBS) was purchased from Gibco. [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) was purchased from Energy Chemical Co. (Shanghai, China).

2. Characterization

¹H NMR spectroscopy was performed on a Bruker Avance NMR spectrometer (400 MHz) with CDCl₃ and DMSO-*d*₆ as solvents and 0.5% tetramethylsilane as internal standard. Gel permeation chromatography (GPC) was carried out on Tosho Silica HLC-8320GPC instrument with DMF as mobile phase. Dynamic light scattering (DLS) study was performed on a Malvern Zetasizer Nano ZS (UK) at room temperature. Transmission electron microscopy (TEM) image was obtained on a Tecnai G2 F20 transmission electron microscope. UV-vis spectroscopy was performed on a Hitachi U3900 Ultraviolet absorption spectrometer.



Figure S1. ¹H NMR spectrum of monomer MAC in CDCl₃.



Figure S2. ¹H NMR spectrum of mPEG-PMAC in DMSO-*d*₆.

The polymerization degree of mPEG-PMAC was calculated by comparing the integral areas of peak b to peak e.



Figure S3. GPC traces of mPEG and mPEG-PMAC.

Table S1 Molecular weights of polymers.

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Polymers	$M_{ m n}{}^{ m a}$	$M_{\mathrm{n}}{}^{\mathrm{b}}$	$M_{ m w}/M_{ m n}{}^{ m b}$
mPEG ₂₀₀₀	2000	2600	1.15
mPEG-PMAC	4400	8000	1.75

a. Calculated by NMR; b. Determined by GPC.



Figure S4. Acid-base titration curve of mPEG-PC solution.



Figure S5. Size and zeta potential distributions of different drug loaded nanoparticles.



Figure S6. The fluorescence spectra and standard curves of DOX (A, C) and Ce6 (B, D). (For standard curves DOX: $\lambda_{ex} = 480 \text{ nm}, \lambda_{em} = 585 \text{ nm}; \text{ Ce6: } \lambda_{ex} = 400 \text{ nm}, \lambda_{em} = 687 \text{ nm})$



Figure S7. The ¹H NMR spectra of DNPs in $D_2O(A)$ and DMSO- $d_6(B)$.



Figure S8. Size changes of DNPs in 10% FBS.



Figure S9. Zeta potentials of DNPs in PBS with different pH values (n = 3).



Figure S10. The relative fluorescence intensity after different irradiation times of SOSG with DNPs at pH 7.4 and 5.0. The mixture of SOSG and DNPs were pre-incubated in PBS for 6 h.



Figure S11. The cytotoxicity of mPEG-PC against NIH/3T3 (A) and 4T1 (B) cells after a 24 h incubation with (+L) or without (-L) light irradiation.

Samples	50% inhibition	75% inhibition	90% inhibition	Γ^a
DNPs	0.334	0.275	0.249	0.980
Ce6+DOX·HCl	0.178	0.184	0.209	0.946

Table S2. Combination index (CI) values of PDT (Ce6) and Chemotherapy (DOX).

a. The linear correlation coefficient of the median-effect plot.



Figure S12. The cytotoxicity of DNPs against 4T1 cells with different irradiation times. The

incubation time was 24 h.



Figure S13. The intracellular DOX release from DNPs by co-localization study.



Figure S14. The drug release of Ce6 with and without light irradiation at pH 7.4. The light irradiation (660 nm, 3 min) was given at 4, 8, and 12 h.

Laser irradiation (min)	0.5	1	3
DNPs concentration (µg/mL)	9.22	6.60	5.58
Ce6 (µg/mL)	1.046	0.749	0.633
DOX (µg/mL)	0.628	0.449	0.380

Table S3. IC_{50} s of DNPs against 4T1 cells with different irradiation times.



Figure S15. H&E analysis of main organs after treatment.