Supplementary Information

A Copper-loaded Self-assembled Nanoparticle for Disturbing the Tumor Redox Balance and Triple Anti-tumor Therapy

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Supplementary Methods, Figures, and Table

Methods

The synthesis of PDMAEMA-PHB-PDMAEMA:

For the synthesis of PDMAEMA-PHB-PDMAEMA, DMAEMA, Br-PHB-Br, CuBr and HMTETA (a molar feed ratio, 54:1:1:1.2) were added into a flask containing a magnetic stirrer and dioxane. After the reactant dissolved completely, the mixture was degassed. Then CuBr was added under nitrogen atmosphere and further purged with nitrogen. The flask was sealed and stirred at 45°C under nitrogen atmosphere. The reaction was stopped and diluting with THF at the appropriate time. And the copolymer was precipitated in hexane to remove DMAEMA monomer. It was then reprecipitated in diethyl ether and dried under vacuum.

Figures

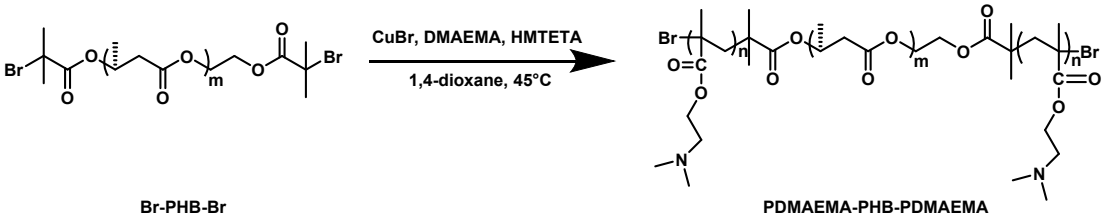

Figure S1. Chemical structures and synthetic route of PDMAEMA-PHB-PDMAEMA.

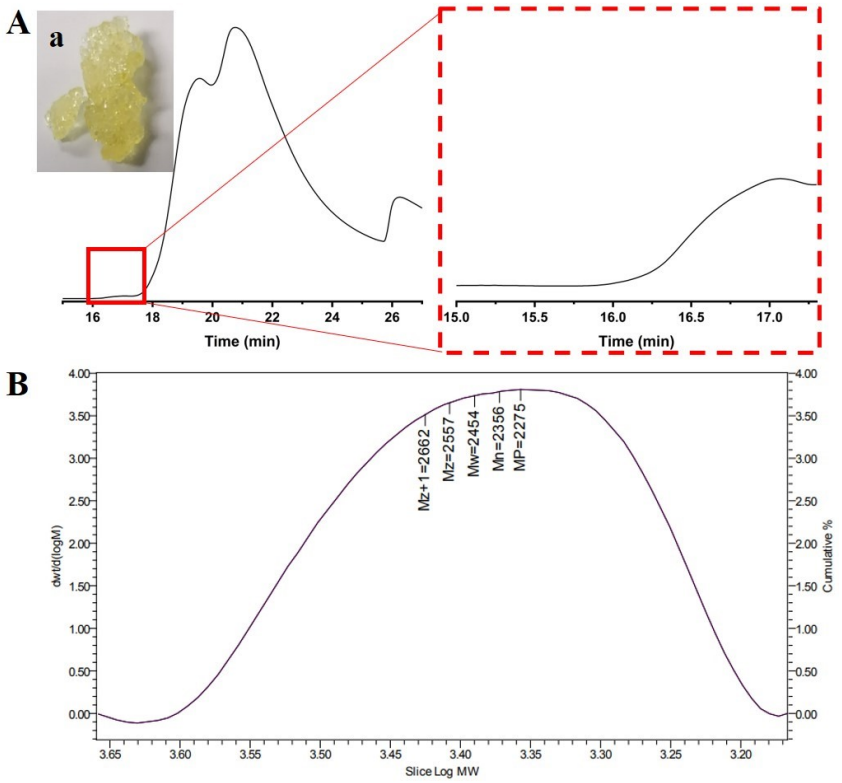

Figure S2. Characterization of PDMAEMA-PHB-PDMAEMA. (A) The GPC elution curve (a: photograph of PDMAEMA-PHB-PDMAEMA after dried in vacuo.). (B) The molecular parameters of PDMAEMA-PHB-PDMAEMA.

The molecular weight of the used Br-PHB-Br is 2120 Da, as reported by Loh *et al*[1]. Surprisingly, the molecular weight of the triblock polymer as determined by GPC testing was only marginally higher than that of Br-PHB-Br, as depicted in **Fig. S2**. But the ^{1}H NMR spectrum of PDMAEMA-PHB-PDMAEMA displayed distinct resonance signals at specific

positions (**a**, **b**, **c**, **d**, **e**, **f**, and **g**), confirming its identity as a triblock polymer (**Fig. S3**). And there are no vinyl bonds in the NMR results. Based on the PHB peak of the methane proton at 5.26-5.23 ppm and the PDMAEMA peak at 4.06-4.05 ppm (**Fig. S3**), the molecular weights and composition of the block copolymers were calculated and summarized in **Table 1**. Specifically, the peak intensity ratio between positions b (methylene) and g (methine) is approximately 16 to 1, indicating an 8 to 1 ratio of PDMAEMA to PHB. This suggests a speculated molecular weight of the triblock copolymer to be 27240 Da. The composition of PHB and PDMAEMA is 7.78% and 92.22%, respectively.

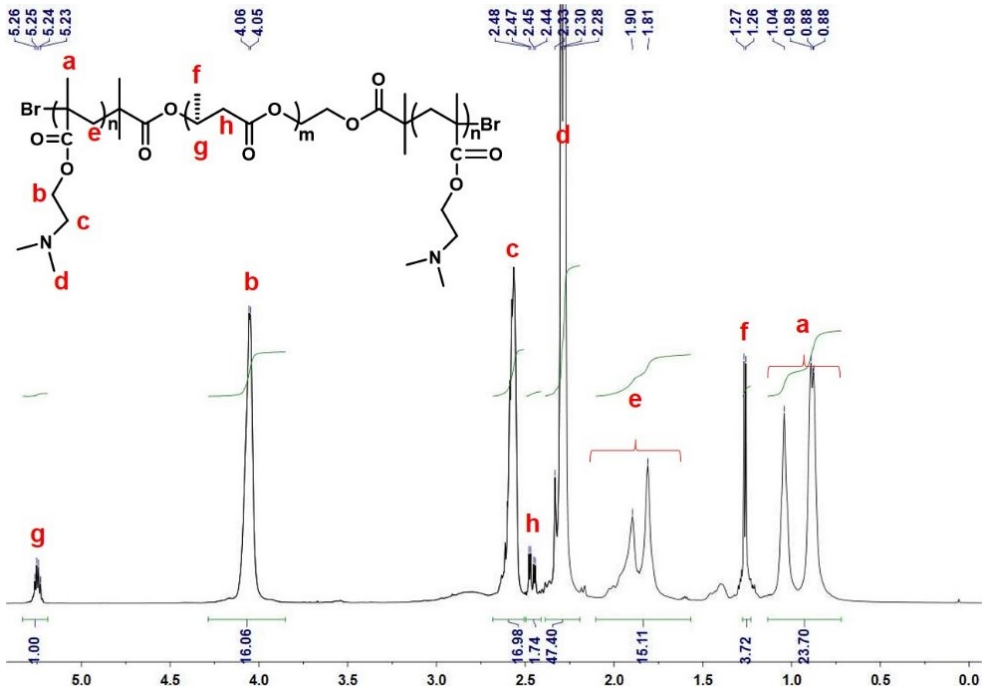

Figure S3. ^{1}H NMR spectrum of the PDMAEMA-PHB-PDMAEMA triblock copolymer.

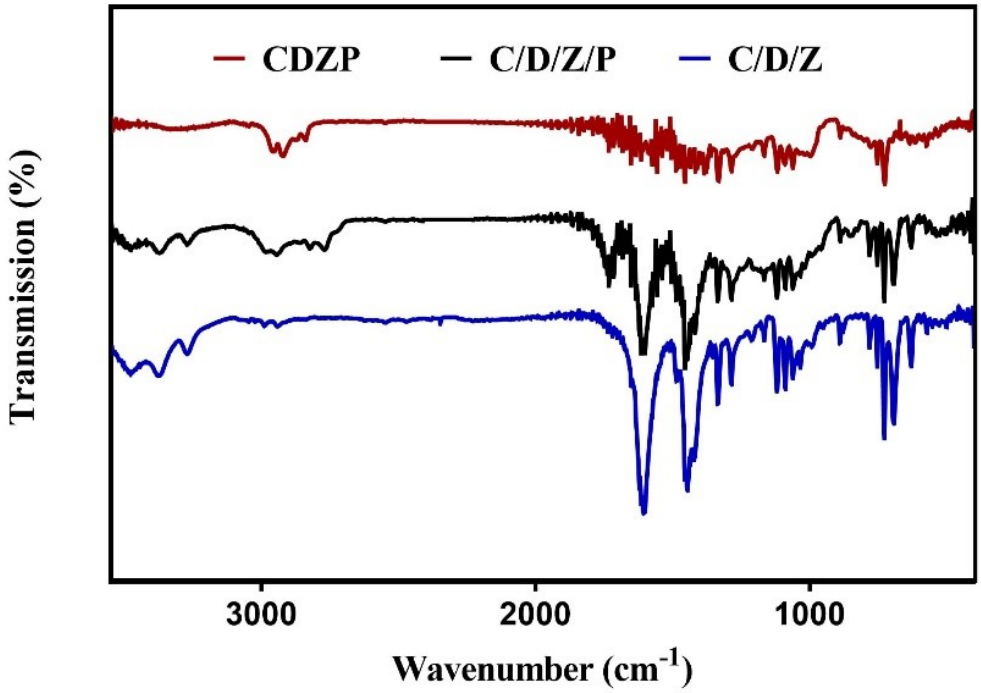

Figure S4. FTIR spectrum of C/D/Z, C/D/Z/P and CDZP.

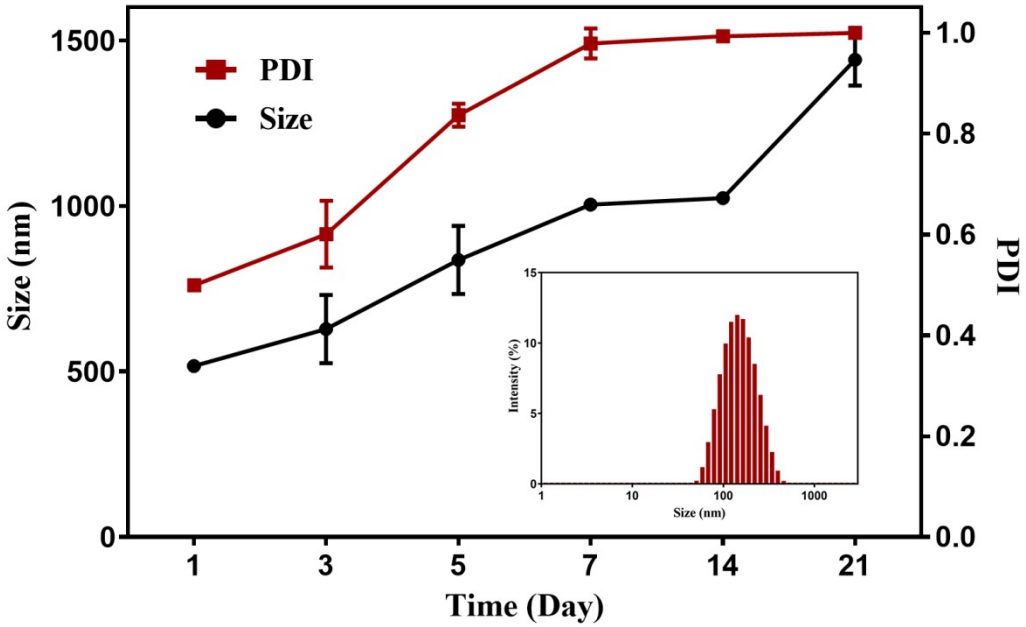

Figure S5. Change of particle size and PDI of CDZ in 21 days (*n*=3).

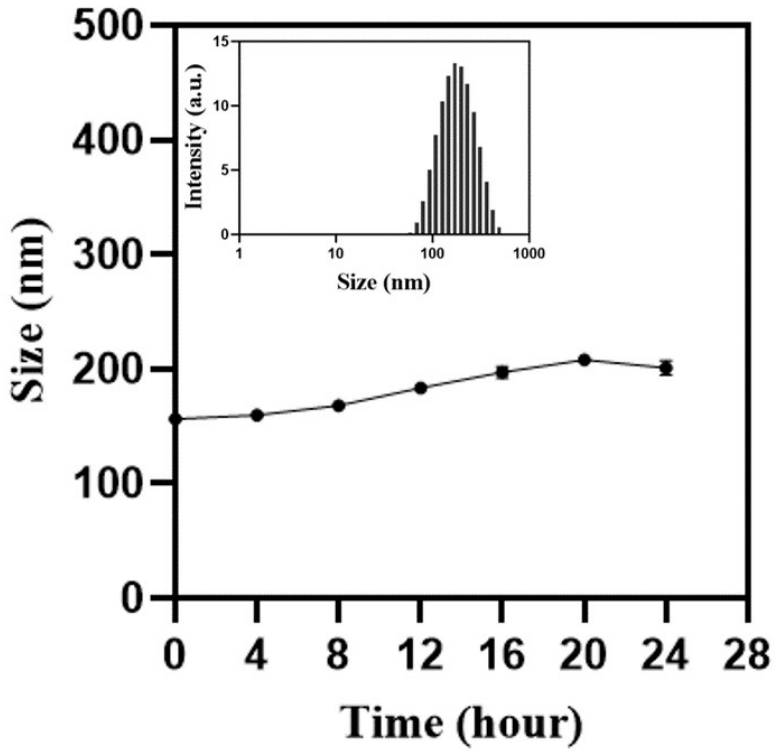

Figure S6. The stability of CDZP NPs in DMEM with 10% FBS.

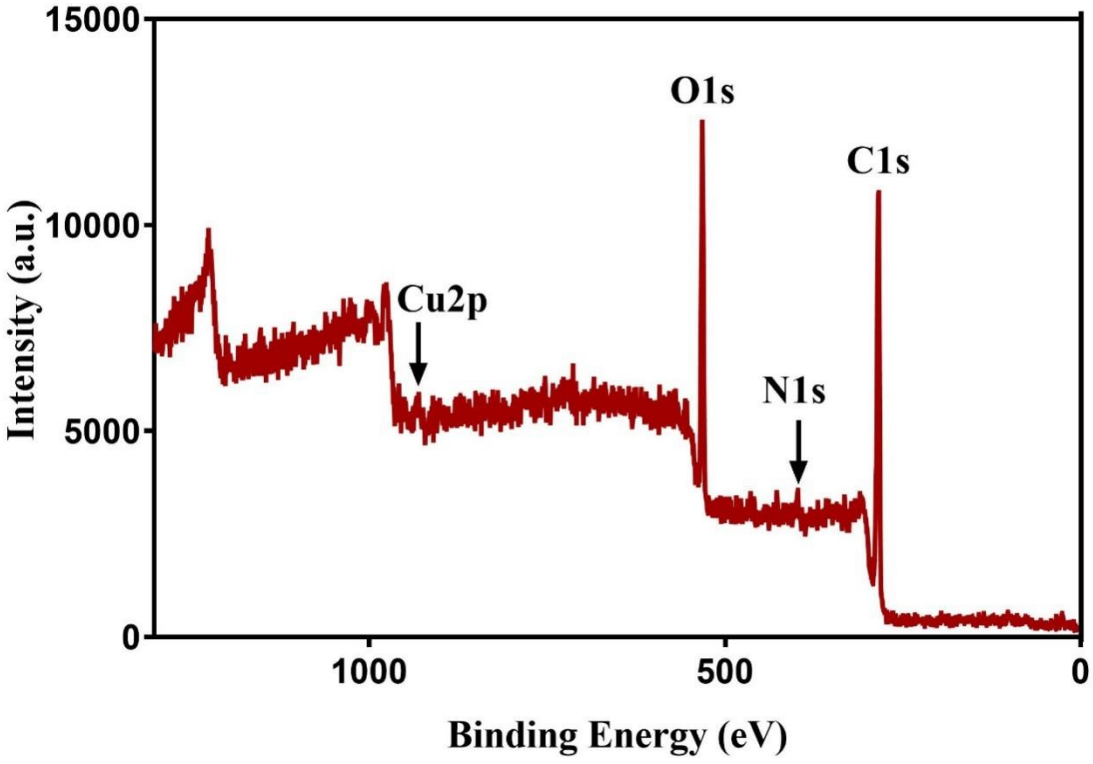

Figure S7. XPS spectrum of CD.

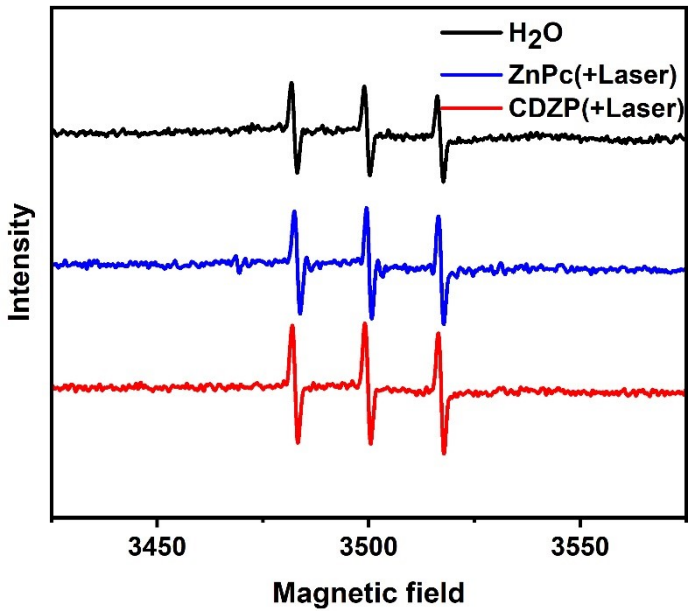

Figure S8. $^1O_2$ generation detected by ESR.

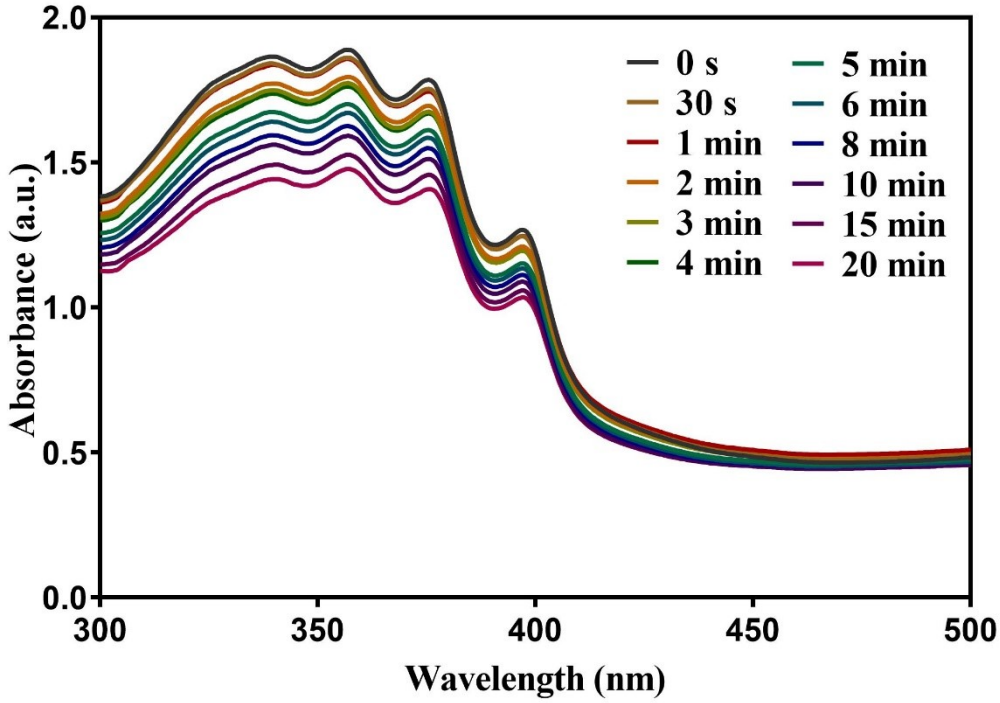

Figure S9. UV-vis spectrum of ABDA co-incubated with CDZP+GSH at different laser irradiation times.

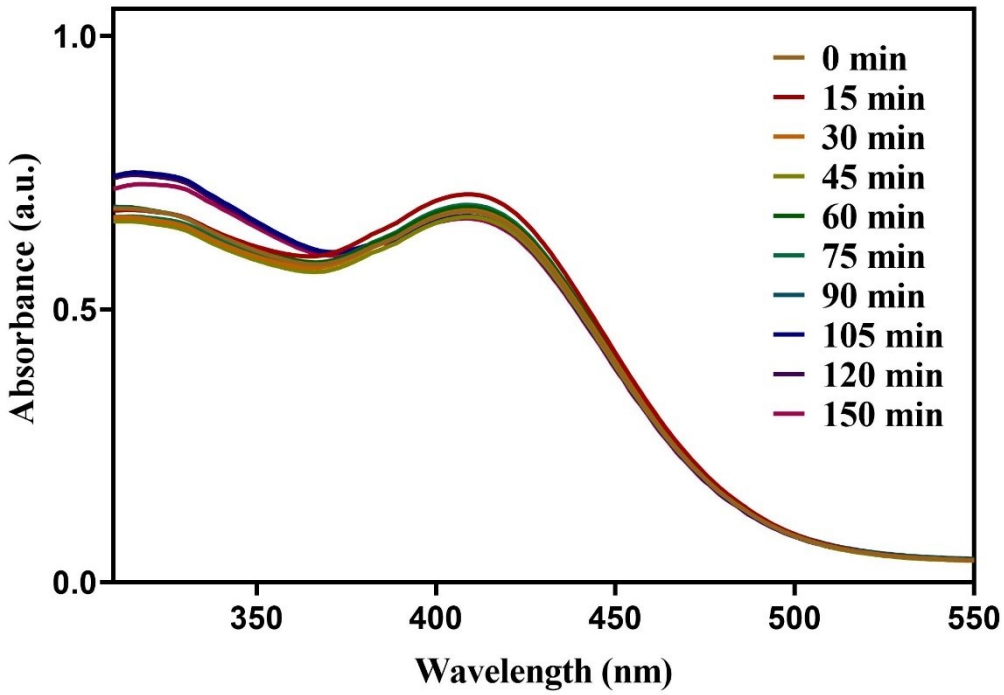

Figure S10. UV-vis spectrum of DTNB co-incubated with GSH.

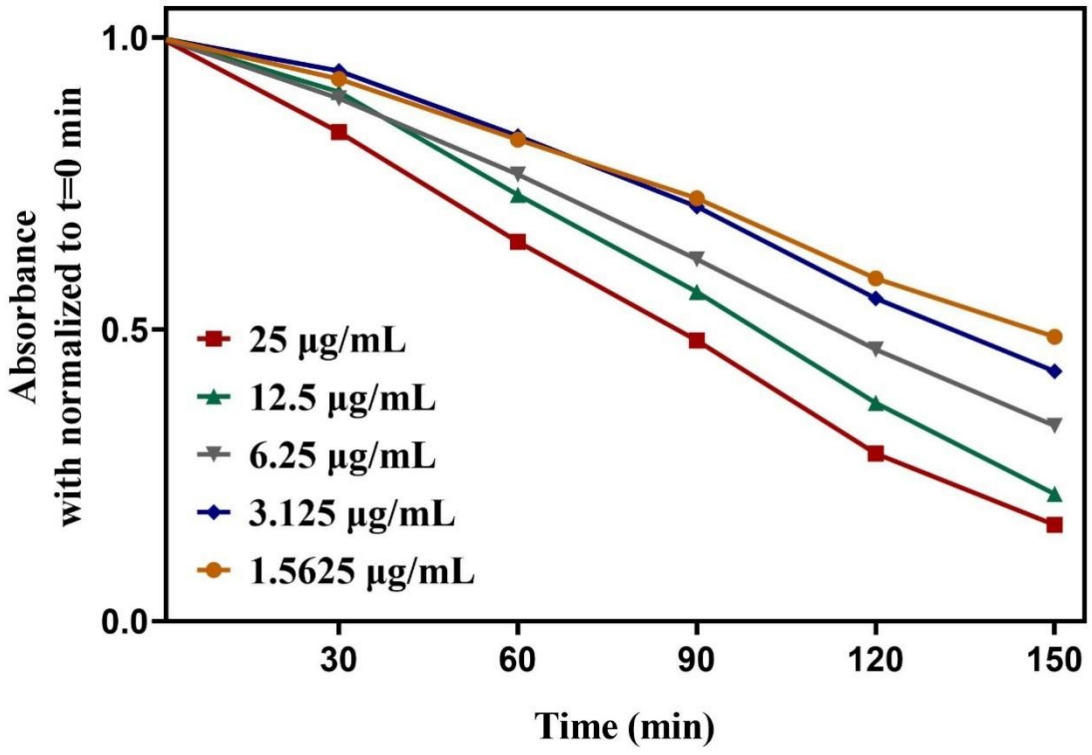

Figure S11. DTNB absorbance of CDZP normalized to t=0 min under different incubation

times.

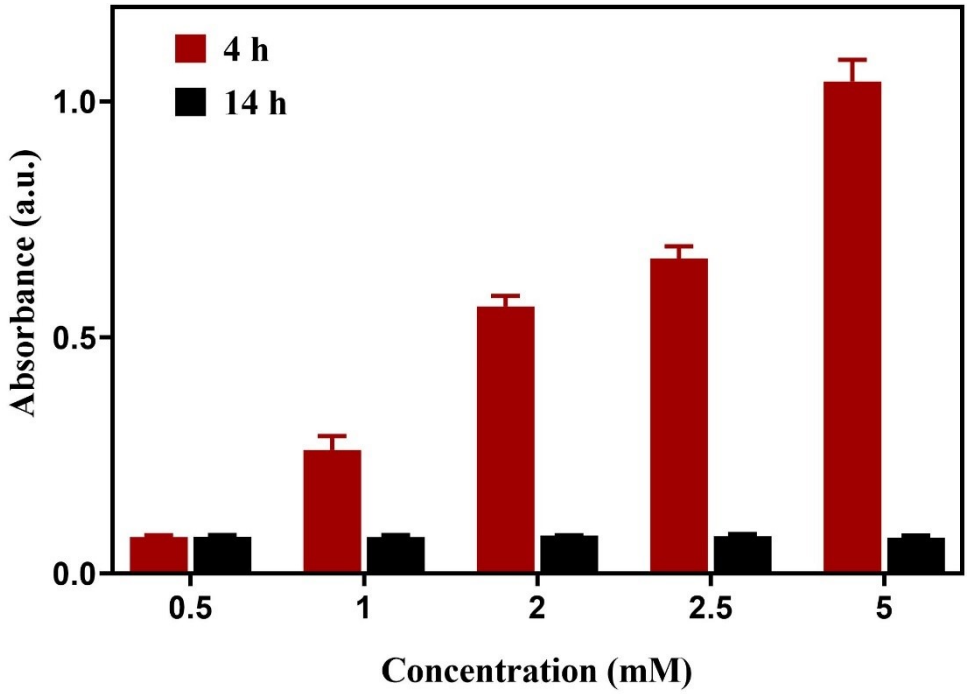

Figure S12. Absorbance of DTNB co-incubated with CDZP NPs and GSH at different concentrations for 4 and 14 h (*n*=3).

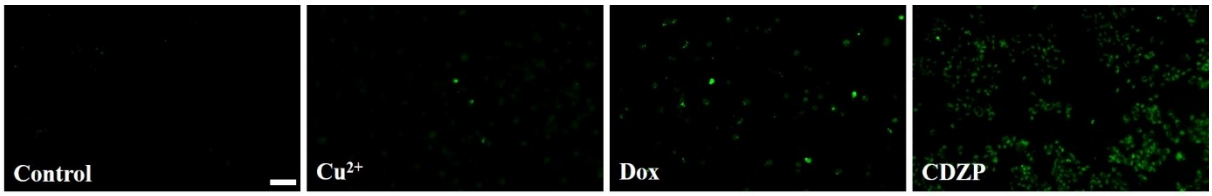

Figure S13. 3-CCA stained Hela cells exposed to different treatments (Scale Bar: 100 μm).

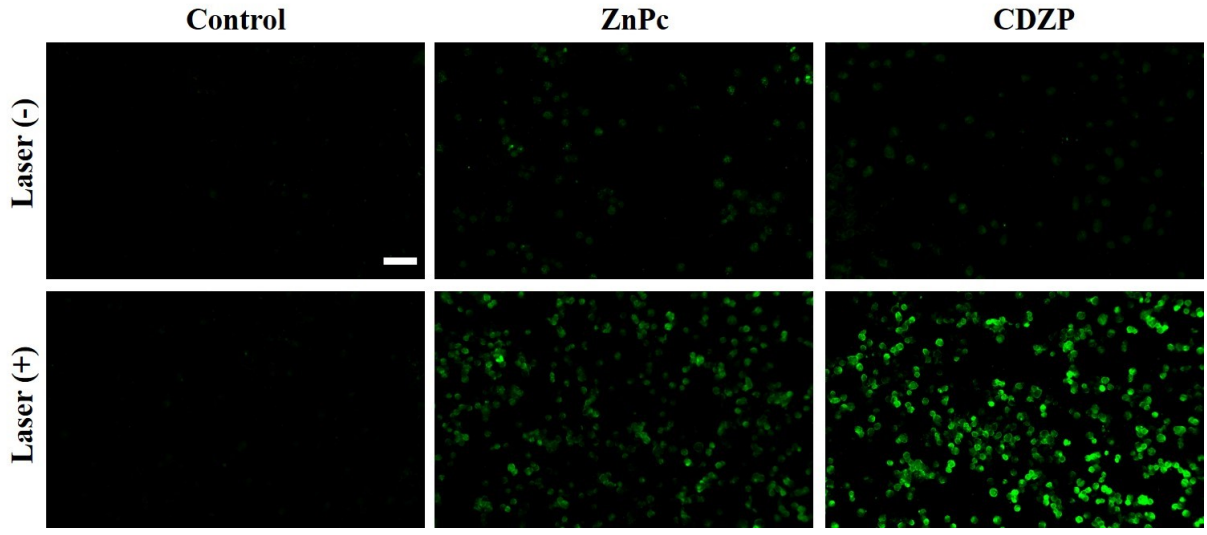

Figure S14. SOSG stained Hela cells exposed to different treatments (Scale Bar: 100 μm).

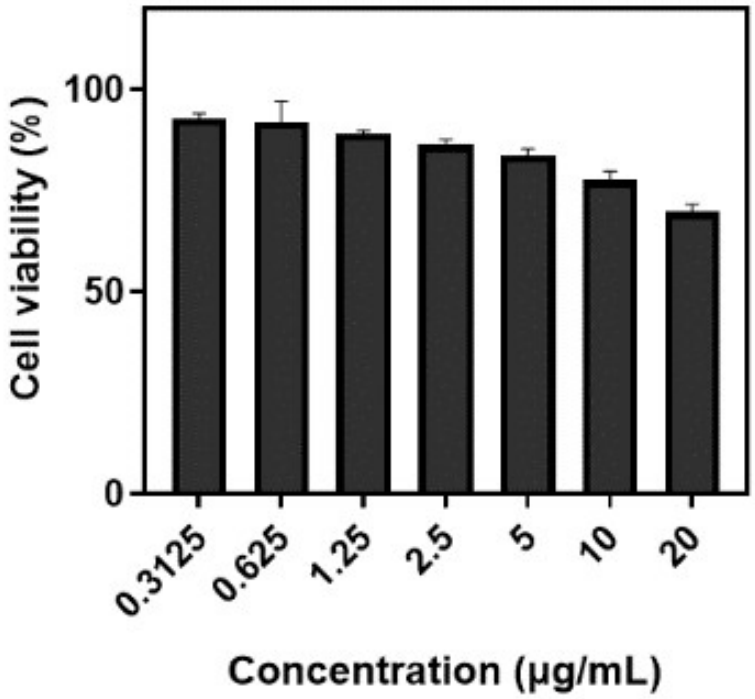

Figure S15. Cytotoxicity assay of CDZP NPs toward L 929 cells.

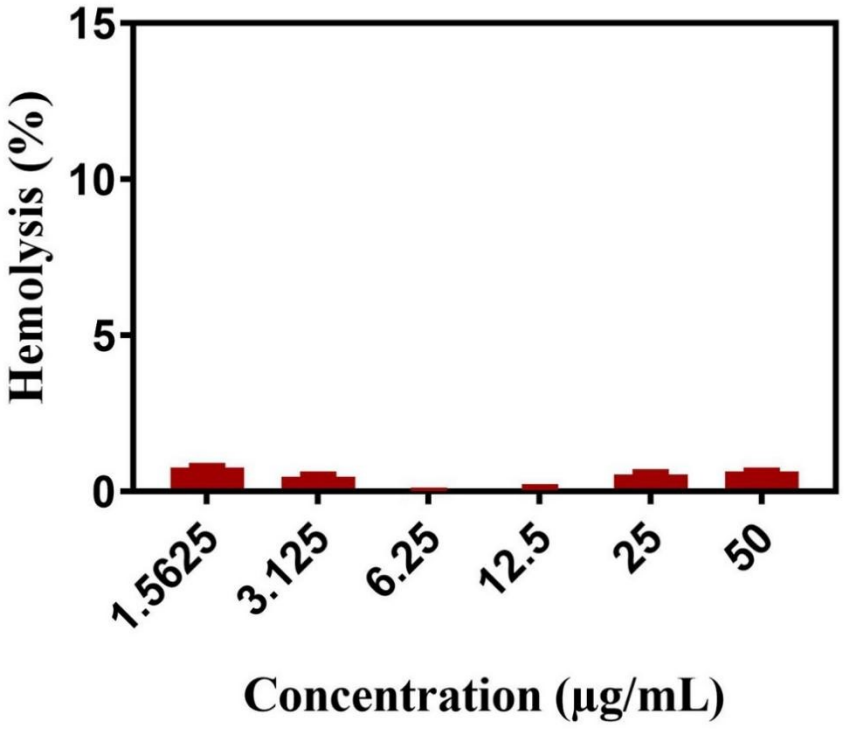

Figure S16. Hemolysis rate of CDZP NPs at different concentrations (*n*=3).

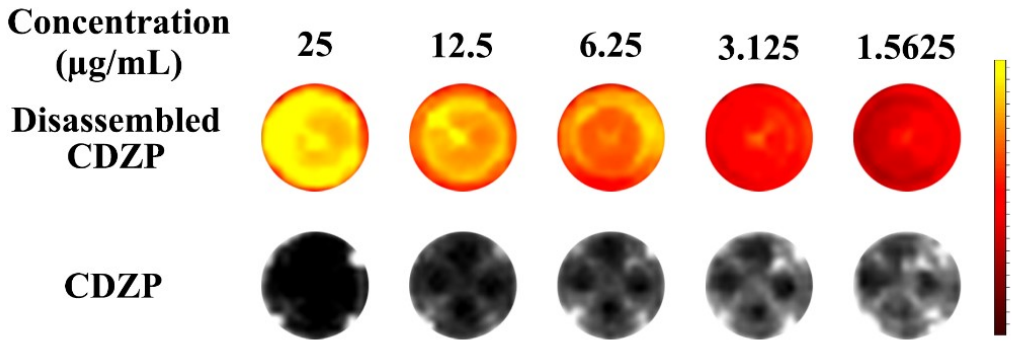

Figure S17. Fluorescence signal intensity of CDZP NPs before and after disassembly at different concentrations.

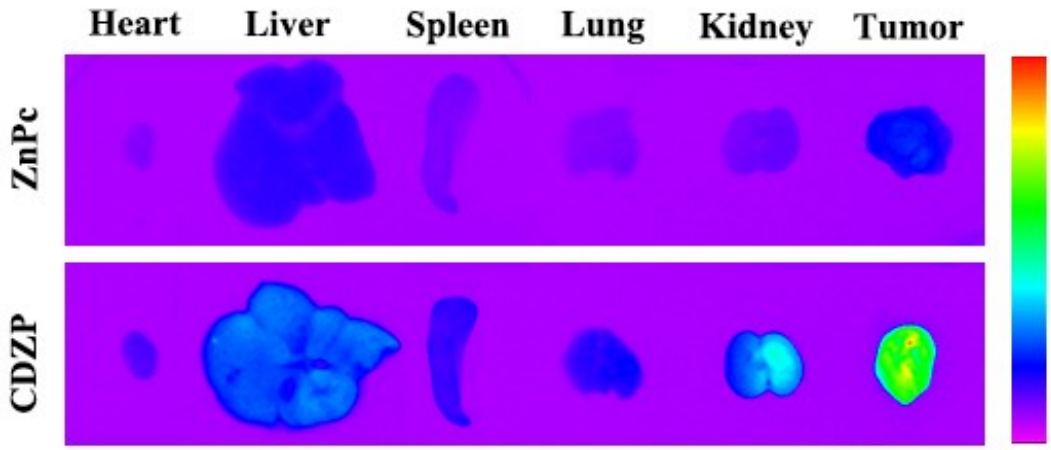

Figure S18. Fluorescence images of major organs and tumors of mice after intravenous injection of ZnPc or CDZP NPs.

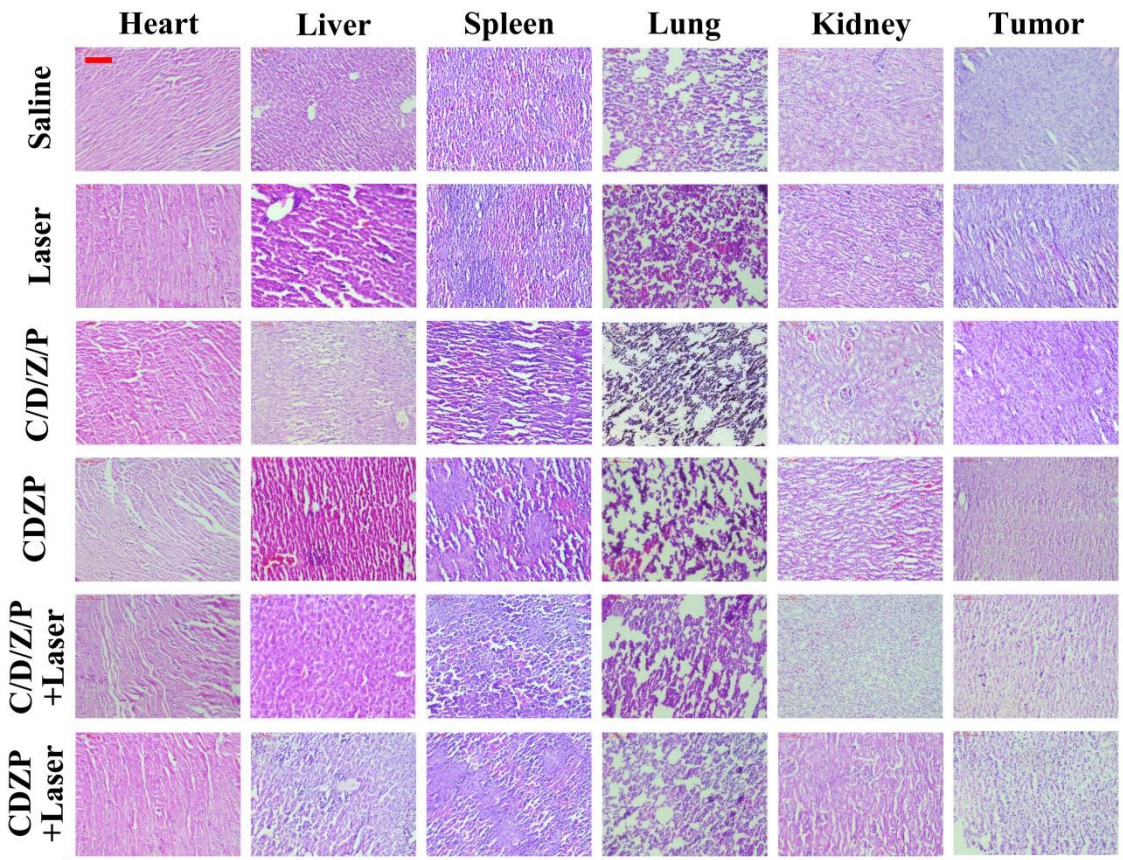

Figure S19. H&E staining histological images of main organs and tumors with different treatments (Scale bar: 100 μm).

Table

Table S1. The molecular parameters of PDMAEMA-PHB-PDMAEMA.

Mn [a]	PDI [a]	Mn [b]	Copolymer composition [b] of PHB (wt%)	Copolymer composition [b] of PDMAEMA (wt%)
2356	1.042	27240	7.78	92.22

[a] Determined by GPC. [b] Calculated from ^{1}H NMR spectrum.

Reference

1. X.J. Loh, S.J. Ong, Y.T. Tung, H.T. Choo, *Polymer Chemistry.* 2013, **4**,