Supporting Information

Self-assembled pH/enzyme dual-responsive prodrug with PEG deshielding for multidrug-resistant tumor therapy

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Supplementary Figures

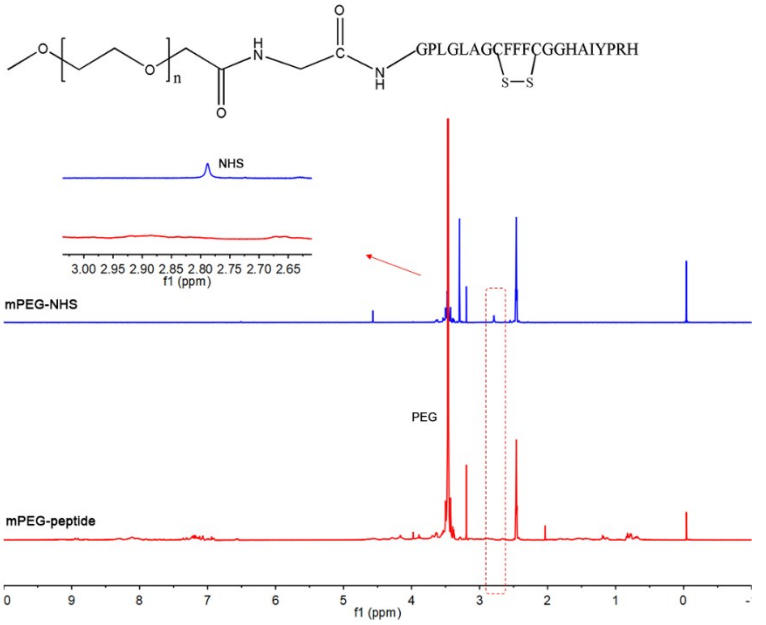

Figure S1. The ^{1}H NMR spectra of peptide and mPEG-peptide in DMSO-*d6*.

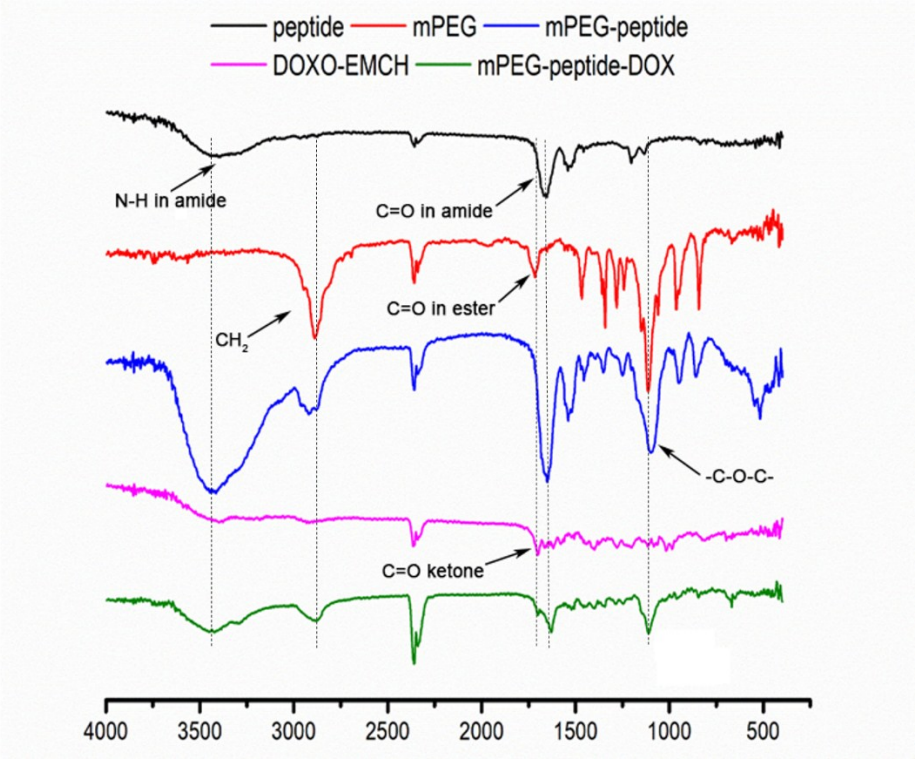

Figure S2. The IR spectra of peptide, mPEG, mPEG-peptide, DOXO-EMCH, and mPEG-peptide-DOX.

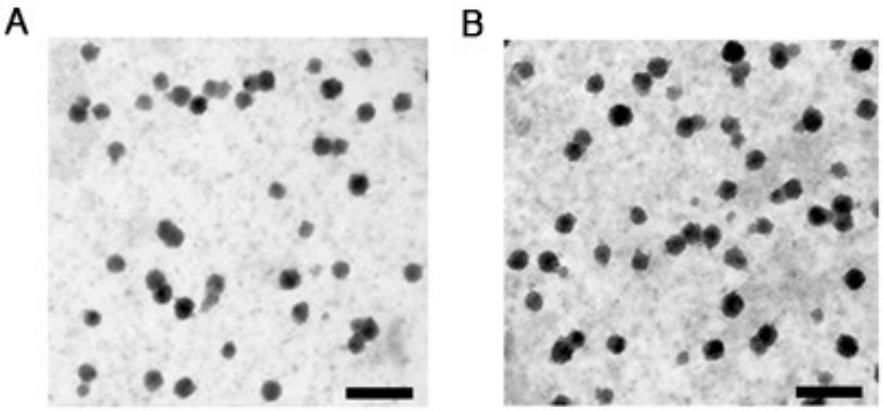

Figure S3. The TEM images of MISNPs before (A) and after (B) treatment with MMP-2. Scale bar was 200 nm.

Supplementary Methods

S1. Chemicals and reagents

Peptides (GPLGLAGCFFFCGGHAIYPRH (MMP-2 sensitive), CGGHAIYPRH (T10), GGGPALLCFFFCGGHAIYPRH (MMP-2 insensitive)) were developed by Sangon Biotechnology Co., Ltd. (Shanghai, China). DOXO-EMCH was purchased from Adooq Bioscience (Irvine, USA) with a purity of 98%. mPEG-NHS (MW 2,000 Da) was obtained from Jiankai (Beijing, China). D-(+)-mannitol and N-(2-hydroxyethyl) piperazine-N′-(2-ethane sulfonic acid) (HEPES) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trypan blue was obtained from Generay Biotech Co., Ltd. (Shanghai, China). RPMI-1640 Medium and Dulbecco's Modified Eagle Media (DMEM) and fetal bovine serum were obtained from Invitrogen (Burlington, ON, Canada). Penicillin was supplied by CSPC Zhongnuo Pharmaceutical Co., Ltd. (Shijiazhuang, China). Streptomycin was obtained from Merro Pharmaceutical Co., Ltd. (Dalian, China). MMP-2 protease was from R&D System. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) was purchased from Sigma. Phosphate-buffered saline (PBS) was from Beyotime Institute of Biotechnology (Jiangsu, China). Ethylenediaminetetraacetic acid (EDTA), potassium hydroxide, sodium bicarbonate, and hydrochloric acid were from

Sinopharm Chemical Reagent Company (Shanghai, China). Acetonitrile and methanol were HPLC grade and from ROE. Formic acid (FA) was purchased by Xilong Chemical Industrial Factory Co., Ltd (Shantou, China). Water was purified and deionized using a Milli-Q system from Millipore (Bedford, MA, USA).

S2. Cell culture

MCF-7 (ATTC, Manassas, VA) and MCF-7/ADR (Keygen Biotech, Nanjing, China) cells were cultured in DMEM and RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C and 5% CO_2 respectively. To maintain a highly drug-resistant cell population, MCF-7/ADR cells were periodically reselected by growing them in the presence of 1 μg/ mL DOX. Experiments were carried out using the cells incubated without DOX for 48 h. Cells were counted with a hemocytometer (Qiujing, Shanghai, China). Cell viability was assessed by trypan blue (0.4%) exclusion, which was completed by mixing cell suspension, trypan blue, and 1 × PBS in a ratio of 2: 5: 3 and counting the percentage of viable cells following a 5 min incubation at 37 °C.