Electronic Supplementary Information (ESI) for Polymeric drug delivery system with actively-targeted cell penetration and nuclear targeting for cancer therapy

Qiaochu Hua[1], *Zhe Qiang*[2], *Maoquan Chu*[3], *Donglu Shi*[4,5], *Jie Ren*[1*]

1. Institute of Nano and Biopolymeric Materials, School of Materials Science and Engineering, Tongji University, Shanghai 201804, China

2. Department of Chemical and Biological Engineering. Northwestern University, Evanston, IL 60208, USA

3. Biomedical Multidisciplinary Innovation Research Institute and Research Center for Translational Medicine at Shanghai East Hospital, School of Life Sciences and Technology, Tongji University, Shanghai 200092, China

4. Materials Science and Engineering Program, Department of Mechanical and Materials Engineering, College of Engineering and Applied Science, University of Cincinnati, Cincinnati, OH 45221-0072, USA

5. Key Laboratory of Basic Research in Cardiology, Ministry of Education, Shanghai East Hospital, Institute for Biomedical Engineering and Nano Science, School of Medicine, Tongji University, Shanghai 200120, China

1. Experimental section

1.1 Materials

Amide-group-decorated poly (ethylene glycol) -poly(ε-caprolactone) (NH_2-PEG_{2000}-PCL_{3400}) was purchased from PengShuoBiochem Co, Ltd (Shanghai, China). Folic acid (FA), 1-Ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydro-chloride (EDC·HCl) and N-hydroxysuccinimide (NHS), Doxorubicin hydrochloride (DOX·HCl, 98%, Adamas-beta), N-Succinimidyl 3- maleimidopropionate (SMP, 98%), 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazo-lium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, USA). Fetal bovine serum (FBS) was purchased from Hyclone. Trypsin-EDTA, penicillin-streptomycin, high-glucose Dulbecco's modified Eagle's medium (DMEM) were purchased from Gibco. CPPs (CKRRMKWKK) was customized by Shanghai GL Biochem Co, Ltd (Shanghai, China). Other common reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). NH_2-PEG_{2000}-PCL_{3400} and CPPs was dried in vacuum at room temperature for 24 h before use while others were used as received without further purification.

A human hepatocellular carcinoma cell line (Huh-7 cells) was received from ZhongShan Hospital (Shanghai, China). Nude mice aged 5-6 weeks were obtained from Shanghai SIPPR-BK Laboratory Animal Co., Ltd. (Shanghai, China) and kept in a specific pathogen-free (SPF) environment.

1.2 Synthesis of FA-PECL polymer

Folic acid (0.44g, 1 mmol), NHS (0.12g, 1 mmol) and EDC·HCl (0.21g, 1 mmol) were dissolved in 100ml DMSO. The mixture was then stirred at 25℃ in dark and nitrogen atmosphere protected overnight. Afterwards, the mixture was added with NH_2-PEG2000-PCL3400 (5.49g, 1 mmol) and drops of triethylamine, and stirred for 24 h. DMSO, as solvent and folic acid unreacted were subsequently removed through dialysis for 3 days in deionized water. The resultant solution was lyophilized and obtained FA-PECL polymer .

1.3 Synthesis of CPP-DOX

1.3.1 Synthesis of SMP-DOX

Firstly, SMP-DOX was prepared by reaction between the 3’ amino group of the daunosamine sugar of DOX and an active ester group of SMP. DOX (43.5 mg, 1 eq). SMP (22.0 mg, 1.1 eq) and triethylamine (TEA, 21 μL, 2 eq) were dissolved in 10 mL of dimethyl sulfoxide (DMSO) and stirred for 3 h, at 25℃ in dark. The resulting solution was precipitated by cold anhydrous diethyl ether (50 mL) and washed three times by anhydrous diethyl ether for further purification. The resultant red solid was separated from solvent by centrifugation and then vacuum dried to obtain SMP-DOX. TEA was used to remove the hydro-chloride salt and maintain basic conditions to favorably generate DOX-SMP.

1.3.2 Synthesis of CPP-DOX

DOX-SMP (7.8 mg, 1 eq) was dissolved in 2 mL DMSO. CPP (13.5mg,1 eq) was dissolved in 2 mL DMSO and 21 μL TEA, and then stirred at 25℃ in dark for 3 h. The

resultant product was precipitated and purified with cold anhydrous diethyl ether and separated by centrifugation.

1.4 Cellular uptake

The Huh-7 cells were seeded at 1.0×105 cells per dish (35 mm) in 2.0 mL of DMEM (containing 10% FBS) at 37℃ for 24 h and incubated for an additional 4 h with FA-PECL/CPP-DOX, PECL/CPP-DOX, FA-PECL/DOX and PECL/DOX micelles with predetermined concentrations, respectively. The Huh-7 cells were subsequently fixed by paraformaldehyde (4%) for 30 min in an incubator and the cell nuclei were stained by DAPI solution. The fluorescence images of Huh-7 cells were taken by a confocal laser scanning microscopy (CLSM, TCS SP, Leica, Germany).

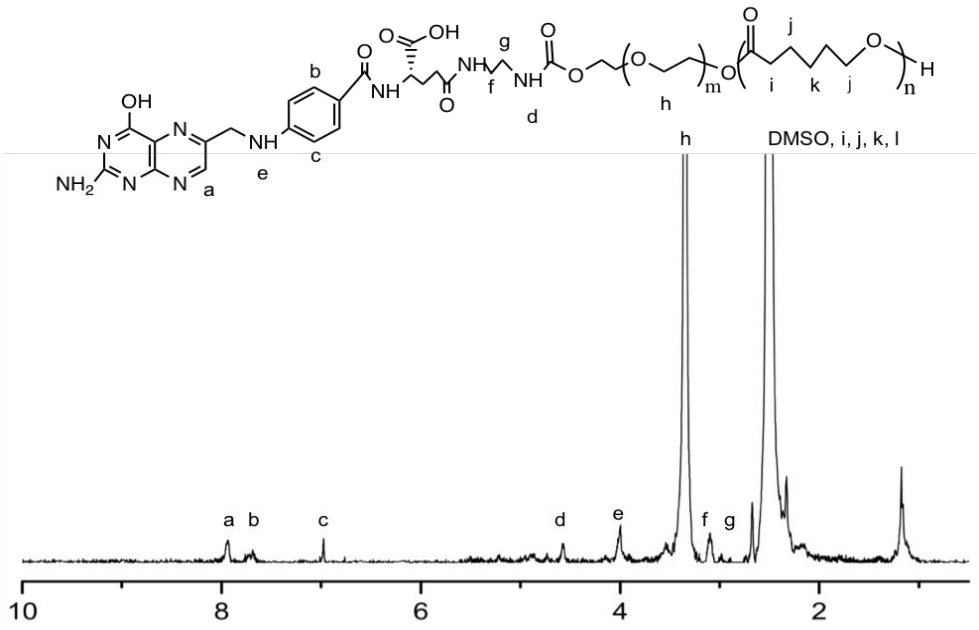

Figure S1. 1H NMR spectra of FA-PEG-PCL

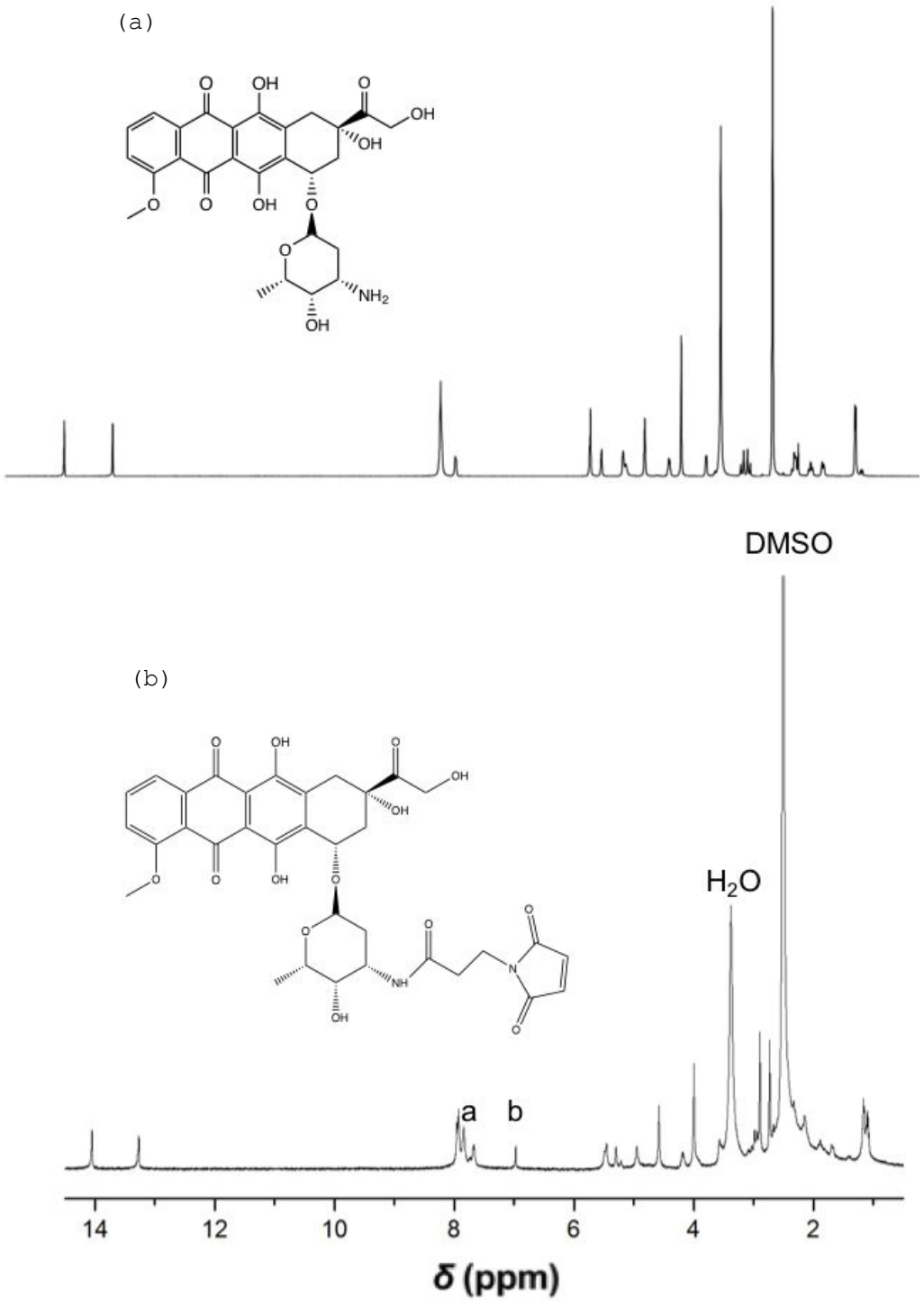

Figure S2. 1H NMR spectra DOX (a), SMP-DOX (b)

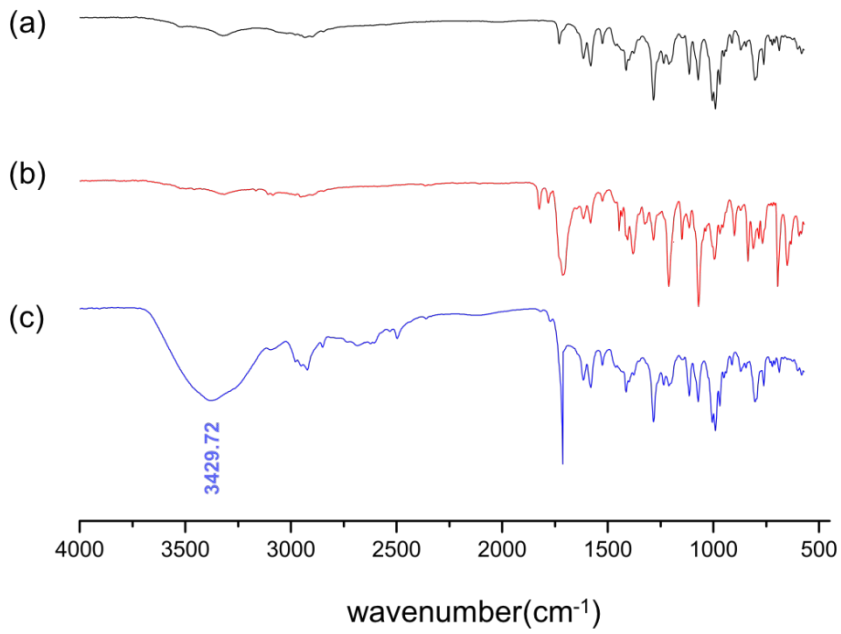

Figure S3. FT-IR spectra of SMP-DOX (a. DOX b. SMP c. SMP-DOX)

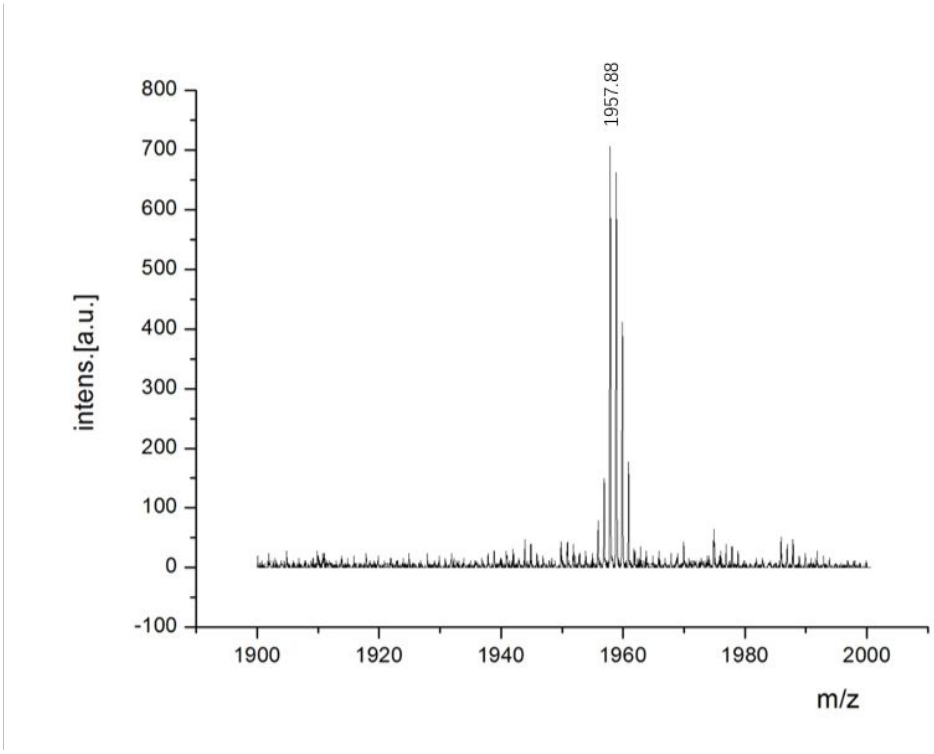

Figure S4. MALDI-TOF spectra of CPP-DOX

Corresponding Author

*E-mail Address: renjie6598@163.com