

Magnetic Cationic Amylose Nanoparticles Used to Deliver Survivin-Small Interfering RNA for Gene Therapy of Hepatocellular Carcinoma In Vitro

Zhuo Wu ^{1,†}, Xiao-Lin Xu ^{2,†}, Jun-Zhao Zhang ^{3,†}, Xu-Hong Mao ⁴, Ming-Wei Xie ¹,
Zi-Liang Cheng ¹, Lie-Jing Lu ¹, Xiao-Hui Duan ¹, Li-Ming Zhang ^{3,4,5,*} and Jun Shen ^{1,*}

¹ Department of Radiology, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou 510120, China; vojoan@hotmail.com (Z.W.); xie_class@126.com (M.-W.X.); czl198601@163.com (Z.-L.C.); lujiejingsysu@163.com (L.-J.L.); duanxiaohui-128@163.com (X.-H.D.)

² Department of Ultrasound, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou 510120, China; xlin200398@163.com

³ Department of Polymer and Materials Science, School of Chemistry, Sun Yat-sen University, Guangzhou 510275, China; zhjunzh3@mail2.sysu.edu.cn

⁴ School of Materials Science and Engineering, Sun Yat-sen University, Guangzhou 510275, China; m13929581729_1@163.com

⁵ Key Laboratory for Polymeric Composite and Functional Materials of Ministry of Education, Guangdong Provincial Key Laboratory for High Performance Polymer-based Composites, Key Laboratory of Designed Synthesis and Application of Polymer Material, Sun Yat-sen University, Guangzhou 510275, China

* Correspondence: ceszhlm@mail.sysu.edu.cn (L.-M.Z.); shenjun@mail.sysu.edu.cn (J.S.);
Tel.: +86-20-8411-2354 (L.-M.Z.); Tel./Fax: +86-20-8133-2702 (J.S.)

† These authors contribute equally to this work.

Supplementary Materials

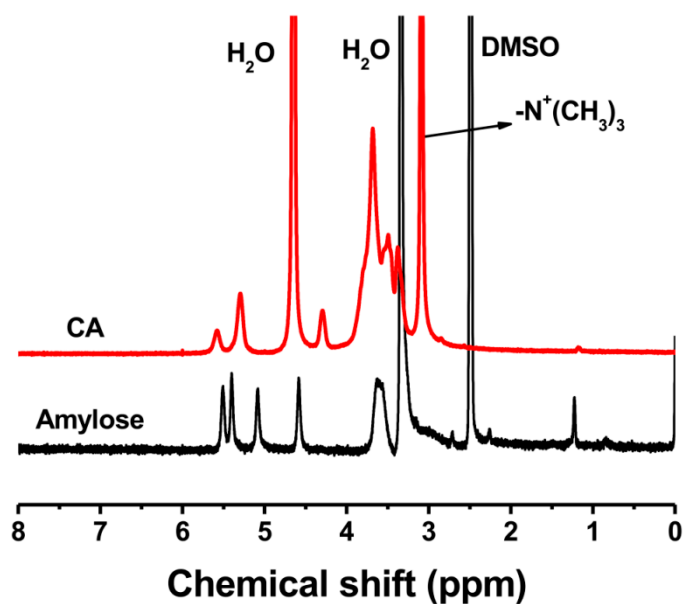


Figure S1. ¹H NMR spectra of amylose and cationic amylose (CA). DMSO:dimethyl sulphoxide. CA showed new peak at 3.09 ppm compared with amylose, which could be assigned to the -N⁺(CH₃)₃.

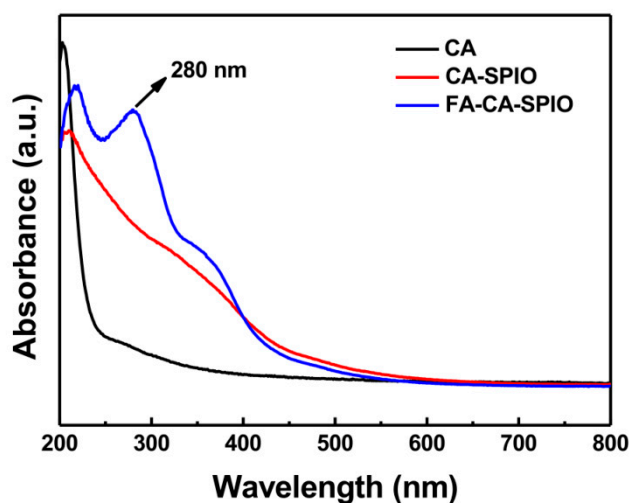


Figure S2. Ultra violet (UV)-vis spectra of cationic amylose (CA), superparamagnetic iron oxide nanoparticle-loaded CA(CA-SPIO) and folate-functionalized CA-SPIO (FA-CA-SPIO). Obvious ultraviolet absorption peak of 280 nm of FA-CA-SPIO compared with CA and CA-SPIO suggests the successful conjugation of folic acid to the quaternized amylose.

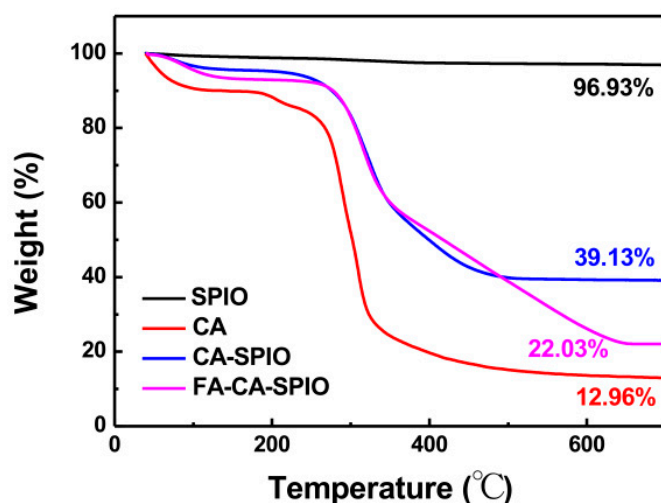


Figure S3. Thermogravimetric (TG) curves of superparamagnetic iron oxide nanoparticles (SPIO), cationic amylose (CA), SPIO-loaded CA(CA-SPIO) and folate-functionalized CA-SPIO (FA-CA-SPIO). Unmodified SPIO has a mass loss of 3.07%. The mass loss of CA, CA-SPIO and CA-FA-SPIO were respectively 87.04 %, 60.87 % and 77.97 %, which indicates the part of SPIO in CA-SPIO was 31.17% and SPIO in CA-FA-SPIO was 10.80%.

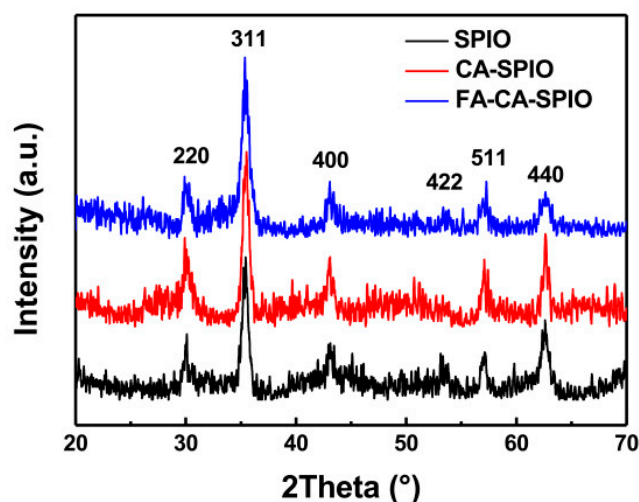


Figure S4. X-ray diffraction diagrams of superparamagnetic iron oxide nanoparticles (SPIO), SPIO-loaded cationic amylose (CA-SPIO) and folate-functionalized CA-SPIO (FA-CA-SPIO). The characteristic peaks at $2\theta = 30.1^\circ$, 35.5° , 43.1° , 53.4° , 57.0° and 62.6° for SPIO, which were marked respectively by their indices (220), (311), (400), (422), (511) and (440), were also observed for CA-SPIO and FA-CA-SPIO.

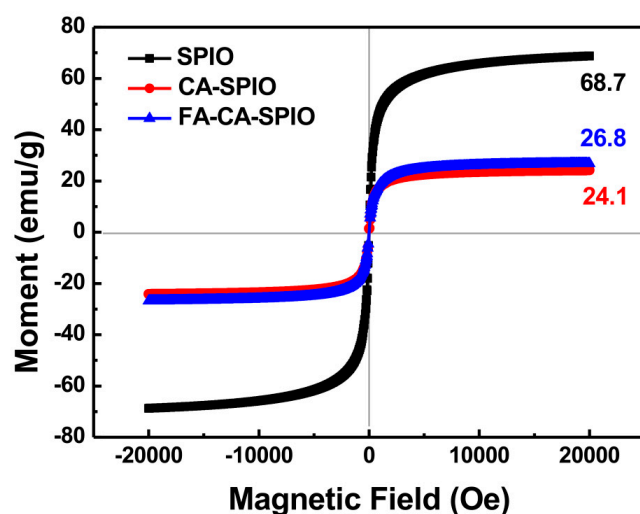


Figure S5. Magnetization curve of superparamagnetic iron oxide nanoparticles (SPIO), SPIO-loaded cationic amylose (CA-SPIO) and folate-functionalized CA-SPIO (FA-CA-SPIO). The saturation magnetization was 68.7 emu/g for SPIO, 26.8 emu/g for CA-SPIOs and 24.1 emu/g for FA-CA-SPIO. CA-SPIO or CA-FA-SPIO remained the excellent magnetic responses.

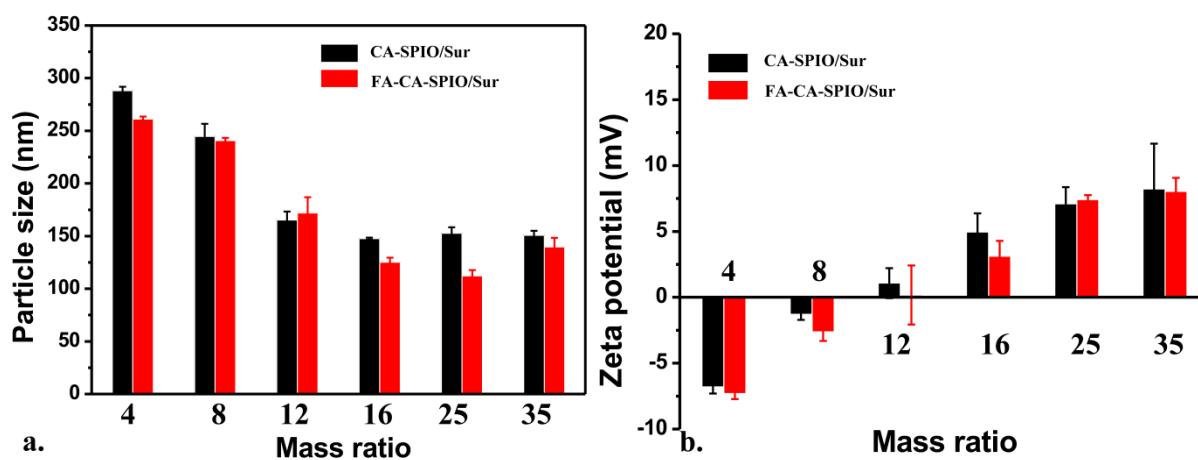


Figure S6. The particle size (a) and zeta potentials (b) of the superparamagnetic iron oxide nanoparticles (SPIO)-loaded cationic amylose (CA-SPIO) and folate-functionalized CA-SPIO (FA-CA-SPIO) complexed with survivin siRNA (Sur) formed at various w/w ratios. With the increase of CA-SPIO or CA-FA-SPIO content in relation to siRNA, the average size of nanocomplexes gradually decreased, while the positive potential was gradually increased. When the weight ratio reached w/w = 12, the nanocomplex had a weak positive charge and a size of approximately 150 nm.

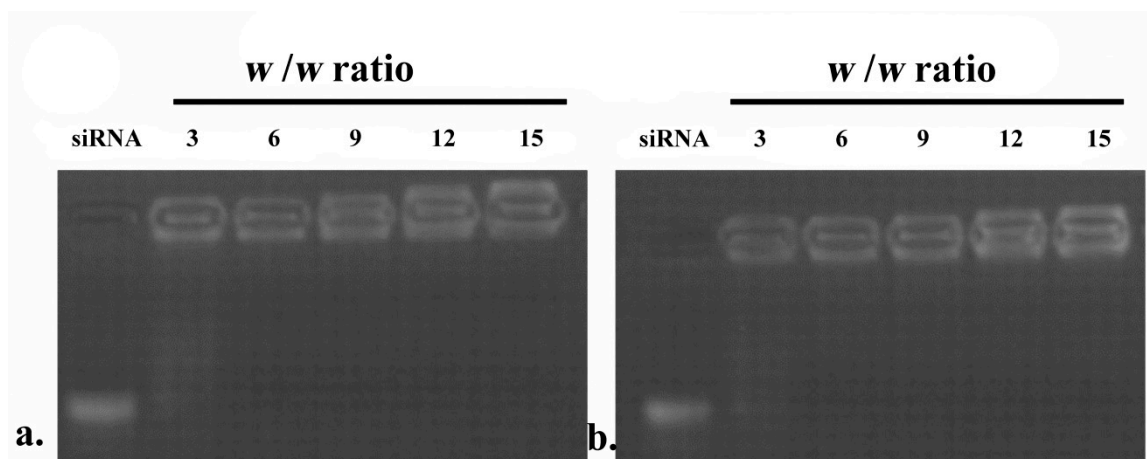


Figure S7. Gel retardation assay of superparamagnetic iron oxide nanoparticles (SPIO)-loaded cationic amylose (CA-SPIO) **(a)** and folate-functionalized CA-SPIO (FA-CA-SPIO) complexed with survivin siRNA (Sur)**(b)**. Amylose nanoparticles were formed at various w/w ratios from 3 to 15. siRNA bands dissociated from nanoparticles were separated by electrophoresis and visualized by an ultra violet (UV) imaging system. Complete siRNA condensation was formed at w/w ratio of 12. Lane 2-7: nanoparticles formed at w/w ratios of 3, 6, 9, 12, and 15; Lane 1: naked siRNA as a control.