Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Smart pH- and Reduction- Dual-responsive Folate-PEG coated Polymeric Lipid Vesicles for Tumor-triggered Targeting Drug Delivery

Sheng Wang, Hanjie Wang, Zhongyun Liu, Liangliang Wang, Xiaomin Wang, Lin Su, Jin Chang*

Institute of Nanobiotechnology, School of Materials Science and Engineering, Tianjin University and Tianjin Key Laboratory of Composites and Functional Materials, Tianjin 300072, PR China

*Corresponding author. Tel: +86 022 87898212; E-mail: jinchang@tju.edu.cn

Synthesis and characterization of PEG with acylhydrazine terminals (PEG-$CH_2CONHNH_2$)

PEG-$CH_2CONHNH_2$ was synthesized according to published procedure. The course of PEG-$CH_2CONHNH_2$ preparation was shown in Fig. S1.[1] Briefly, PEG monomethyl ether (10.4 g) was fully dissolved in toluene (100 mL) and potassium tert-butoxide (2.05 g) dissolved in tert-butyl alcohol (30 mL) was added. Then ethyl bromoacetate (3.2 mL) was added slowly over a period of 30 min. The solution was stirred at room temperature for 24 h, filtered, concentrated with dichloromethane, and precipitated in diethyl ether three times to obtain a white solid product. The precipitate was dried under a vacuum at room temperature to obtain PEG-$CH_2COOC_2H_5$. Subsequently, PEG-$CH_2COOC_2H_5$ (10 g) was dissolved in methanol (100 mL) and a mixture of hydrazine hydrate (30 mL) and methanol (40 mL) was added to the solution dropwise. After reaction for 24 h at room temperature, the solution was filtered. Then most of the methanol solvent was removed under reduced pressure. The concentrated solution was extracted with dichloromethane, dried with anhydrous magnesium sulfate and precipitated with anhydrous diethyl ether. The white precipitate was dried under a vacuum at room temperature to obtain PEG-$CH_2CONHNH_2$.

Fig. S2 depicts the ^{1}H NMR spectrum of PEG-$CH_2CONHNH_2$. As shown in the figure, the peak at δ =8.8 was attributed to hydrazine (-$NHNH_2$),

suggesting the successful prepare of PEG-$CH_2CONHNH_2$.

Characterization of FA-DS

Chemical shifts from folate 6.50-7.50 (phenyl group) is visible in the ^1H NMR spectrum shown in Fig. S3, which corresponded to the aromatic protons of folate, suggesting the successful conjugation of FA to the DS.

Critical aggregation concentration (CAC) characterization of DS

In brief, aliquots of pyrene in THF were added to containers and THF was evaporated. The final concentration of pyrene was 6×10^{-7} M. A total of 3 mL of various concentrations (0 to 1 mg mL^{-1}) of aqueous polymer solutions were added to each container and the solutions were kept at room temperature for 24 h before measurement. Fluorescence spectra were measured at room temperature using a fluorescence spectrofluorometer (F-4500, Japan) with a slit width of 2.5 nm for emission. Excitation wavelength was set at 338 nm, and excitation spectra were recorded ranging from 360 to 500 nm. Spectra were acquired with a scan speed of 240 nm min^{-1}.

Pyrene was chosen as the fluorescent probe because pyrene preferentially solubilized itself into the hydrophobic region of nanoparticles, the fluorescence intensity was greatly affected by the environmental change around pyrene. The fluorescence emission spectra of pyrene incorporated into DS in water at 25 ℃ were measured. The value of I_1/I_3 ratio increased

significantly with the transfer of pyrene molecules from a polar to a more hydrophobic microdomain. The CAC was determined from the intersection point of two straight lines. As shown in Fig. S4, the CAC value of DS was determined as about 38.19 mg L^{-1}.

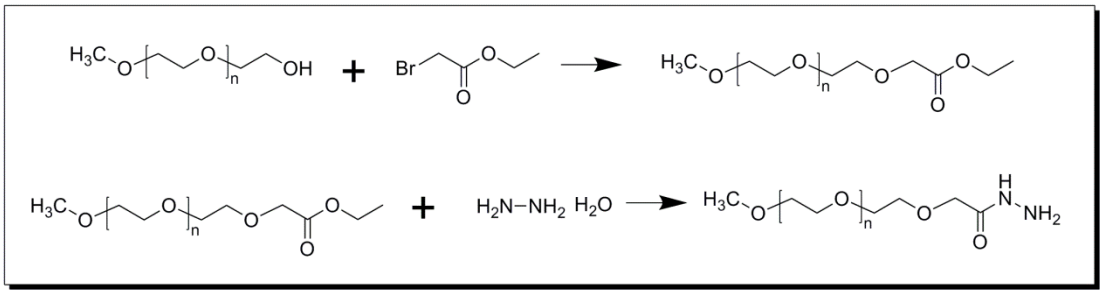

Fig. S1. The synthesis process of PEG-$CH_2CONHNH_2$.

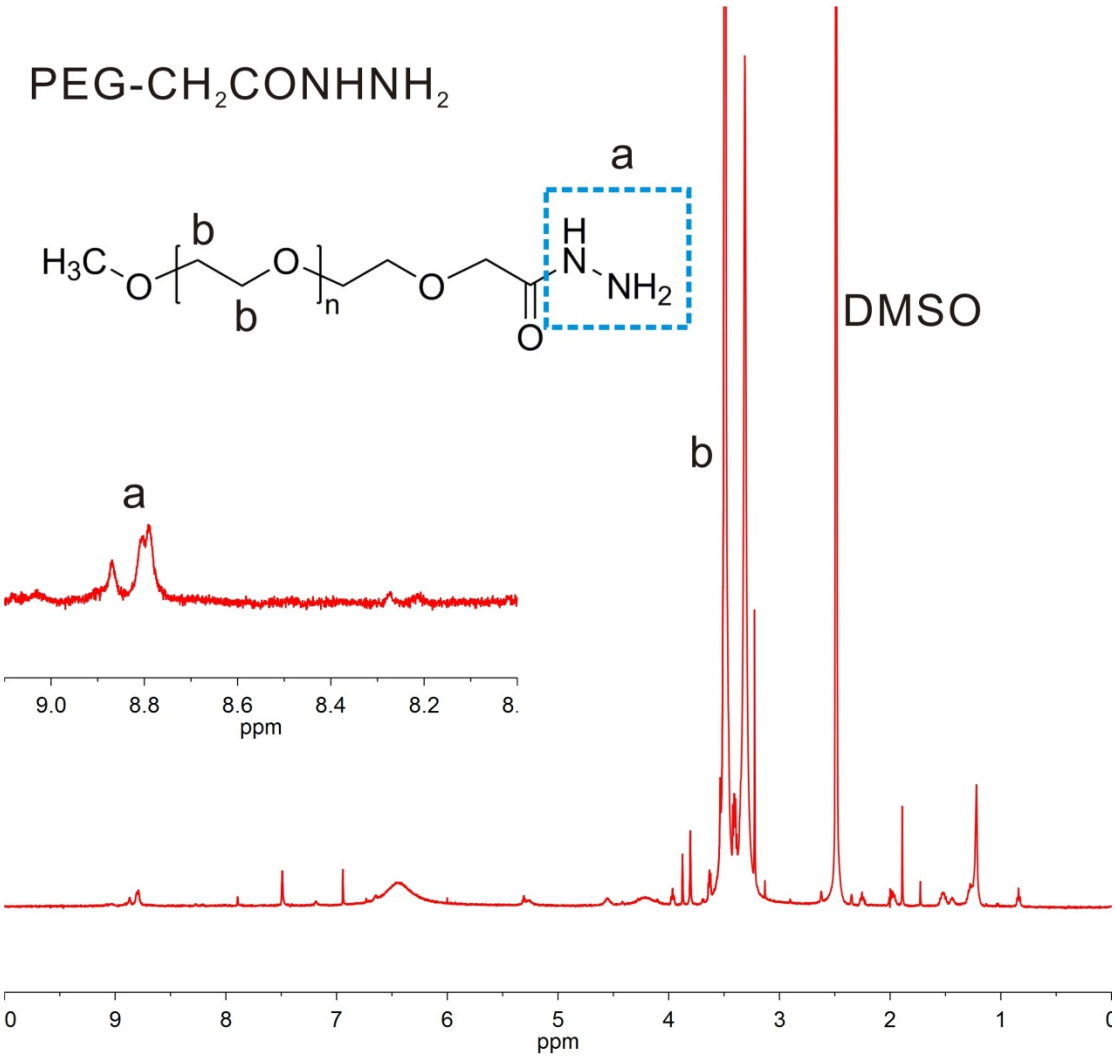

Fig. S2. ^1H NMR spectrum of PEG-$CH_2CONHNH_2$.

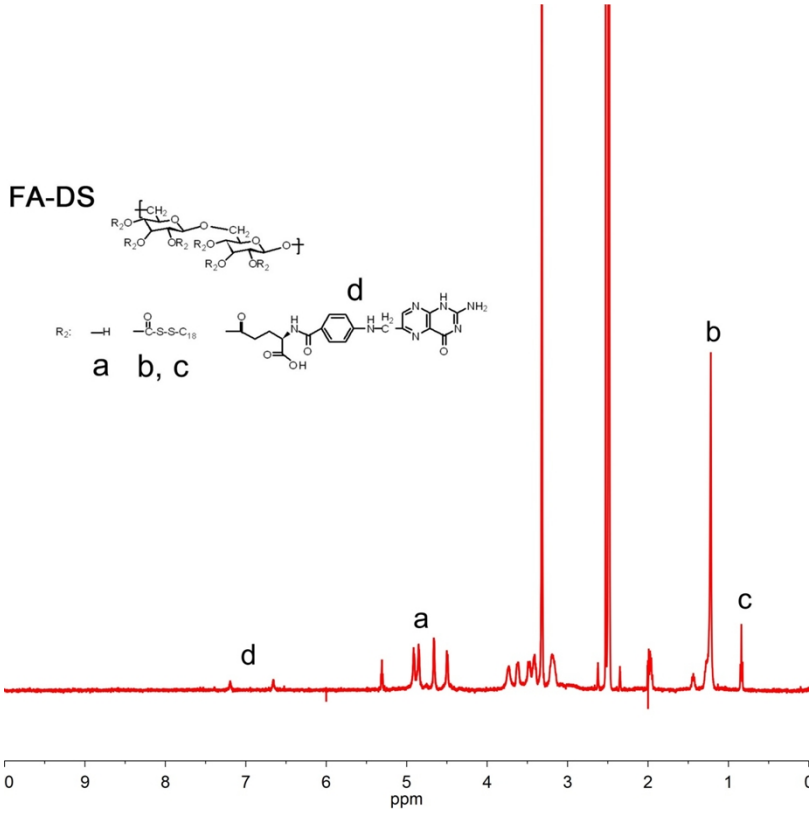

Fig. S3. ^{1}H NMR spectrum of FA-DS.

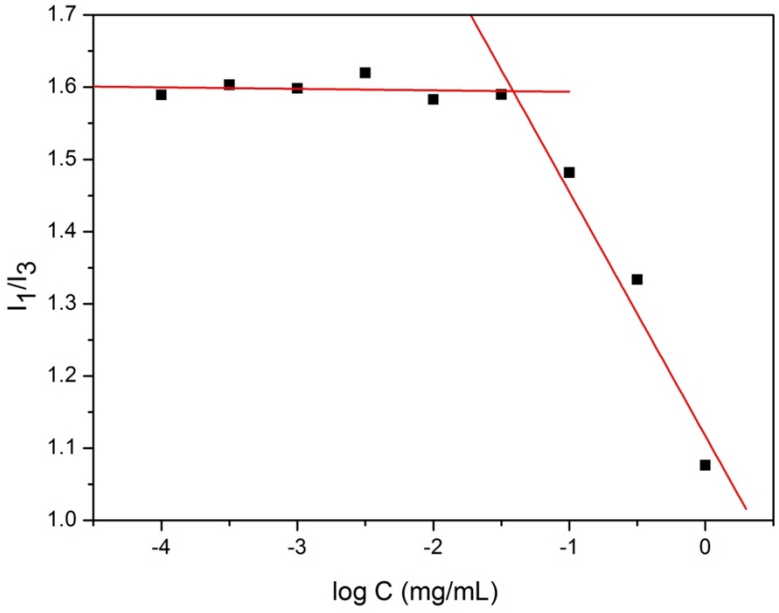

Fig. S4. The change of intensity ratio (I_1/I_3) versus the concentration of DS values.

Reference

1. F. Qiu, C. Tu, Y. Chen, Y. Shi, L. Song, R. Wang, X. Zhu, B. Zhu, D. Yan and T. Han, *Chemistry-A European Journal*, 2010, **16**, 12710-12717.