

Electronic Supplementary Information for pH-sensitive drug delivery system with Aggregation-induced emission feature for cell imaging and intracellular drug delivery

Haibo Wang^a, Gongyan Liu^{bc*}, Haiqi Gao^b, Yunbing Wang^{c*}

^a Textile Institute, College of Light Industry, Textile and Food Engineering, Sichuan University, Chengdu, 610065, China.

^b National Engineering Laboratory of Clean Technology of Leather Manufacture, Sichuan University, Chengdu 610065, China. Email: lgy_3506@163.com

^c National Engineering Research Center for Biomaterials, Sichuan University, Chengdu 610065, China. E-mail: Yunbing.Wang@scu.edu.cn

Materials

Dextran 10, odium periodate, hydrazine hydrate were purchased from Sigma-Aldrich. Zinc powder, benzophenone, 4-Hydroxybenzophenone, TiCl_4 , tert-Butyl bromoacetate, K_2CO_3 were purchased from Aladdin. Methylene dichloride was dried by CaH_2 and THF was refluxed with Na wire before use. Other reagents without being listed were used as received.

Synthesis of TPE-hydrazide

TPE-hydrazide was obtained by a three-step synthesis.

The first step is to synthesize TPE-OH. Under an N_2 atmosphere, benzophenone (18.2 g, 0.1 mol), 4-Hydroxybenzophenone (19 g, 0.1 mol), zinc powder (16 g, 0.24 mmol) and 40 mL THF were added into a three-necked flask equipped with a magnetic stirrer at 0 °C. Then, TiCl_4 (13 mL, 0.12 mmol) was slowly added with the temperature under 10 °C. The mixture stirred at the room temperature for 0.5 h, then reflux overnight. After the mixture cooled to room temperature, THF was concentrated under reduced pressure. Then, 50 mL dilute hydrochloric acid (1 mmol L^{-1}) was added and extracted with DCM. The crude product was purified by a silica gel column, eluting with 10 percent petroleum ether in ethyl acetate. A white solid

was obtained TPE-OH. ^1H NMR (500 MHz, CDCl_3): 7.05–7.14 (m, 9H), 6.9–7.0 (m, 6H), 6.7 (d, 2H), 6.50 (d, 2H).

Then, ethyl bromoacetate (2 g, 10mmol), TPE-OH(3.5 g, 10 mmol), K_2CO_3 (2 g, 15 mmol) and acetonitrile were added into a flask and reflux at 100 °C. After 12h, the resulting mixture solution was separated by filtration. The crude product was purified through silica gel column, eluting with 10 percent petroleum ether in ethyl acetate. A white solid was obtained TPE-Et. ^1H NMR (500 MHz, CDCl_3): 7.05–7.14 (m, 9H), 6.9–7.0 (m, 6H), 6.7 (d, 2H), 6.50 (d, 2H), 4.6 (s, 2H), 4.45 (m, 2H), 1.3 (t, 3H).

Finally, 1 g of TPE-Et was dissolved into methanol. Then, hydrazine hydrate (3 ml) was added and stirred vigorously. After 10 h, the mixture solution was pour into water and extracted with DCM for three times. The collected organic layer was concentrated under reduced pressure. The obtained white solid was TPE-hydrazide. ^1H NMR (500 MHz, CDCl_3): 7.05–7.14 (m, 9H), 6.9–7.0 (m, 6H), 6.7 (d, 2H), 6.50 (d, 2H), 4.45 (s, 2H), 4.3 (s, 2H).

Synthesis of oxidized Dextran

An aqueous solution 5 g of dextran and 2g of sodium periodate was dissolved in 20 mL water at room temperature and stirred for 5 h. Then, the resulting solution was dialyzed for 3 days against water, using a dialysis membrane with a MWCO 3500 Da. After 3 days the solution was lyophilized.

Synthesis of Dextran-hydrazone-TPE

For synthesis of Dextran-hydrazone-TPE, 0.5 g of oxidized Dextran, 0.5 g of TPE-hydrazide and a drop of trifluoroacetic acid were dissolved in 10 mL DMSO and the resulting solution was stirred at room temperature. After for 24 h, the mixture solution was poured into 400 mL ethanol to precipitate out the produce. The separated polymer was purified by dissolving in DMSO and dialyzing against water for 2 days. The resulting solution was lyophilized to give a white powder.

Preparation of blank micelles and DOX-loaded micelles

Micelles were prepared by the dialysis. 10 mg of Dextran-hydrazone-TPE was dissolved in 5 mL DMSO and stirred. After 1h, 5 mL of water was added slowly and stirred for another 2h. Then, the mixture solution was transferred into a dialysis tube

and dialyzed against water for 2 days. DOX-loaded micelles were prepared through the similar method. 10 mg of Dextran-hydrazone-TPE and 3 mg DOX were dissolved in 5 mL DMSO and stirred for 1h. Then 5 mL of water was added slowly and stirred for another 2h. Finally, the solution was dialyzed against water for 2 days. Drug loaded capacity was calculated according with the literature.[1]

In vitro drug release

The release of DOX from drug loaded micelles was studied under different pH media at 37 °C. Briefly, 2 mL of drug loaded micelles solution (1 mg mL⁻¹) was added to a dialysis bag and then immersed into 20 mL PBS media (pH 5.0 or pH 7.4) in a tube. The tubes were kept at 37 °C in a thermostatted incubator with constant shaking (100 rpm). At setting time points, 5 mL media were taken out and replaced with a fresh medium. To confirm the concentration of DOX in different time, the samples were lyophilized and dissolved in methanol. The concentration of DOX in methanol was determined using a luminescence spectrometer with 488 nm excitation and 590 nm emission.

In vitro Cell viability assay

The biocompatibility of Dextran-hydrazone-TPE was evaluated by MTT assays. In brief, HUVEC cells and HeLa cells were seeded in 96-well plates at 5×10^3 cells per well in 200 μ L of culture medium and incubated for 24 h. Then Dextran-hydrazone-TPE was added with the final concentration from 10 to 1000 μ g mL⁻¹ and i cultured for another 48 h. Finally, 20 μ L MTT with the concentration of 5 mg mL⁻¹ was added. After 4h, the medium was removed and washed two times with ater. 150 mL DMSO was added to dissolve the obtained crystals, and the absorbance was measured at a wavelength of 570 nm.

In order to study the cell cytotoxicity of drug loaded micelles, drug loaded micelles were dissolved in culture medium with the final DOX concentrations from 0.1 to 10 mg mL⁻¹. After HeLa cells (5×10^3 per well) were attached on the 96-well plates, the culture medium was replaced with 200 μ L of medium containing drug loaded micelles. The cancer cells were incubated for 48 h and measured with MTT assays.

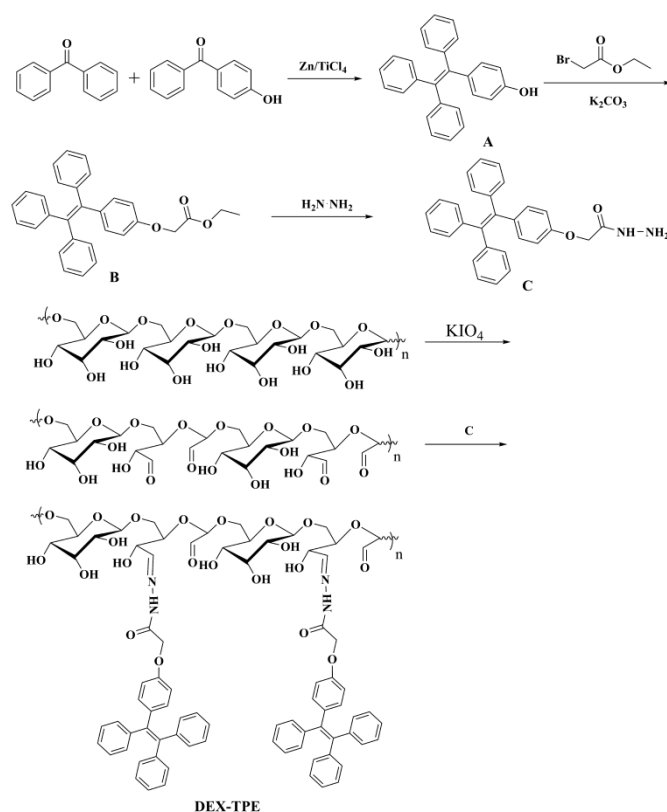
Cellular uptake and cell image

The cell internalization was characterized by fluorescence microscopy and flow cytometry.

Fluorescence microscopy observation. HeLa cells were seeded in confocal dishes at 2×10^4 cells per well and incubated for 24 h. Then, the cells were incubated with drug loaded micelles with the final DOX concentration of $5 \mu\text{g mL}^{-1}$ in fresh culture medium. After being incubated for 1 h or 3 h, the medium was removed and washed three times with cold PBS. Then the cells were fixed with 4% formaldehyde for 15 min and observed using a fluorescence microscope.

Flow cytometry study. HeLa cells (1×10^5 cells/well) were seeded in twelve-well plates and incubated for 24 h. The culture media were withdrawn and drug loaded micelles with the DOX concentration $5 \mu\text{g mL}^{-1}$ in fresh culture medium were added. After incubated for 1 or 3 h, the cells were washed three times with cold PBS. Then, the cells were harvested by trypsin treatment and resuspended in 0.5 mL of PBS for the next detection. 1×10^4 cells were collected using a FACSCalibur flow cytometer.

Results and discussion



Scheme S1 Detailed synthetic route of Dextran-hydrazone-TPE

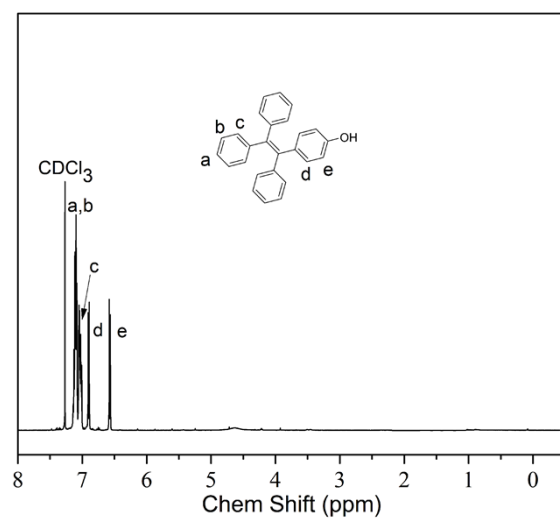


Fig. S1 ¹H NMR spectrum of TPE-OH

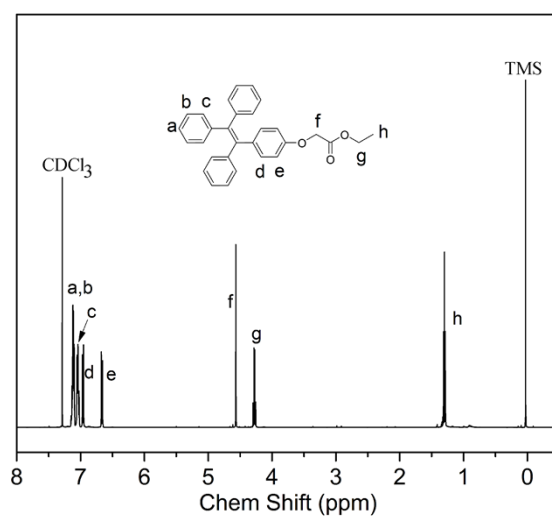


Fig. S2 ¹H NMR spectrum of TPE-Et

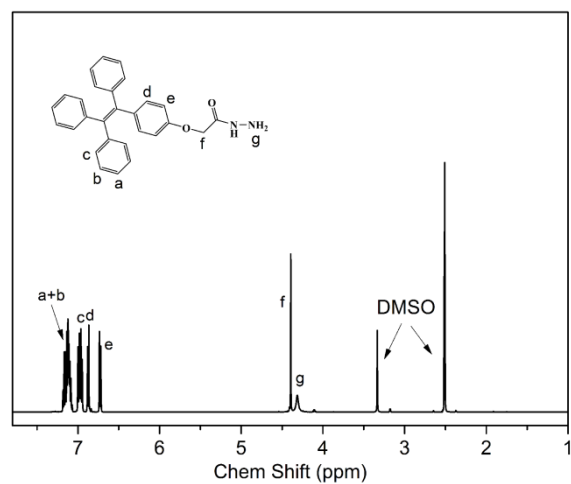


Fig. S3 ^1H NMR spectrum of hydrazone-TPE

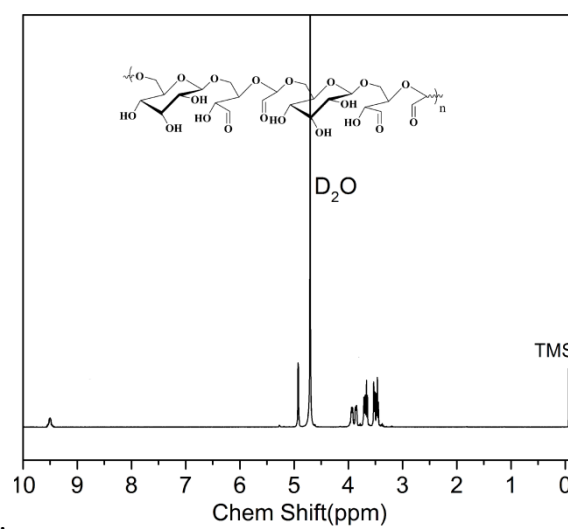


Fig. S4 ^1H NMR spectrum of oxidized Dextran

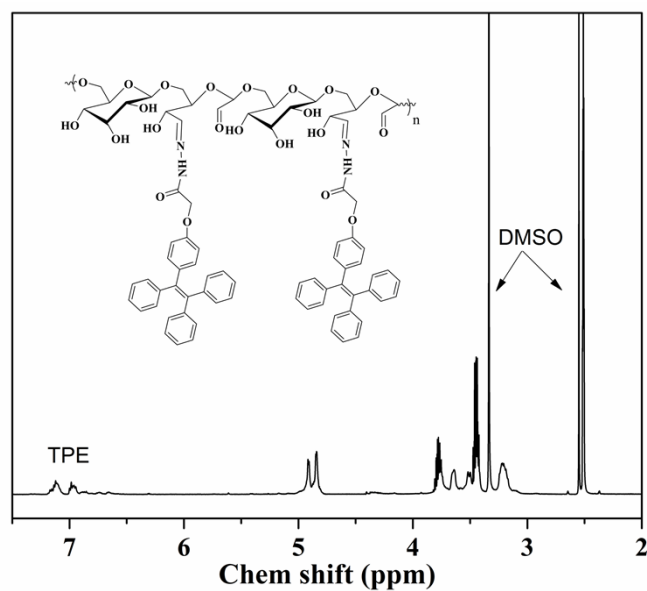


Fig. S5 ^1H NMR spectrum of Dextran-hyd-TPE

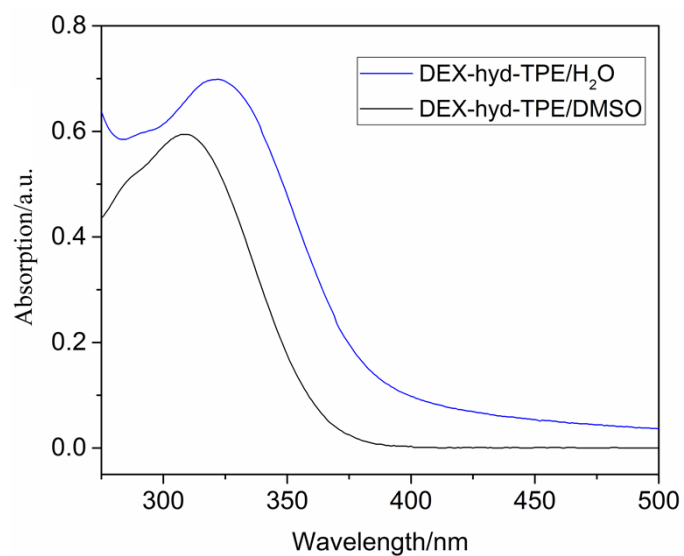


Fig. S6 UV-Vis spectrum of Dextran-hyd-TPE in water or DMSO

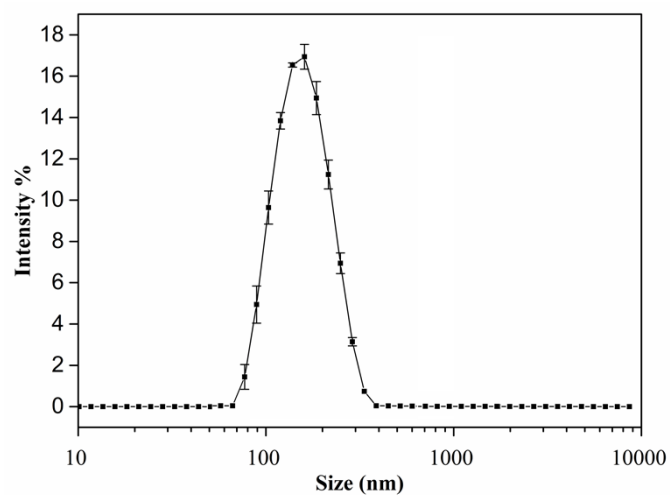


Fig. S7 DLS plots of drug loaded micelles

Notes and references

1 C. Zhang, S. Jin, S. Li, X. Xue, J. Liu, Y. Huang, Y. Jiang, W. Chen, G. Zou and X. Liang, *ACS Appl. Mater. Interfaces*, 2014, **6**, 5212.