Electronic Supplementary Material (ESI) for Nanoscale Horizons.

Supporting Information

Redox stimuli-responsive Hollow Mesoporous Silica Nanocarriers for Targeted Drug Delivery in Cancer Therapy

Ye Tian,[a] Ranran Guo,[a] Yunfeng Jiao,[a] Yangfei Sun,[b] Shun Shen,[c] Yajun Wang,[d,*] Daru Lu,[b] Xingguo Jiang,[c] and Wuli Yang[a,*]

[a] State Key Laboratory of Molecular Engineering of Polymers and Department of Macromolecular Science, Fudan University, Shanghai 200433, P.R. China.

[b] State Key Laboratory of Genetic Engineering and School of Life Sciences, Fudan University, Shanghai, 200433, P. R. China.

[c] Key Laboratory of Smart Drug Delivery, Ministry of Education & PLA, School of Pharmacy, Fudan University, Shanghai 201203, P. R. China.

[d] Department of Chemistry, Fudan University, Shanghai 200433, P.R. China.

* Corresponding author. E-mail: wlyang@fudan.edu.cn; yajunwang@fudan.edu.cn

Experimental Section

Materials and methods

Cetyltrimethylammonium bromide (CTAB), tetraethyoxysilane (TEOS) and aqueous ammonia solution (25 wt%) were purchased from Shanghai Chemical Reagents Company (Shanghai, China). Tert-butyl acrylate (tBA), 2,2'-dithiodipryidine and (3-mercaptopropyl) trimethoxysilane were obtained from Aladdin (Shanghai, China). Acetic acid was purchased from Qiangsheng Chemical Company (Jiangsu, China). Transferrin (Tf), 2-iminothiolane hydrochloride (ITH), 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich. Doxorubicin in the form of hydrochloride salt was obtained from Beijing Huafeng United Technology Company (Beijing, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and other biological reagents were purchased from Invitrogen Corp.

Transmission electron microscopy (TEM) was performed on Tecnai G2 20 TWIN. The hydrodynamic diameters and Zeta potentials of samples were measured by Zetasizer Nano-ZS (Malvern). Thermogravimetric (TGA) data were obtained by a Pyris 1 TGA thermal analyzer with a flow of N_2 from 100 to 800 °C (20 °C min^{-1}). N_2 adsorption−desorption isotherms were obtained on a Micromeritics Tristar 3000 pore analyzer. The loading and release of DOX was measured by UV-vis (Lambda 35). Raman spectra were obtained by XploRA. Fluorescence spectra were measured on FLS 920. Confocal laser scanning microscopy (CLSM) were performed on a Leica, TCS SP2. The flow cytometry analysis was carried on Gallios Flow cytometry. The near infrared fluorescence imaging was performed on In Vivo Xtreme (Bruker).

Synthesis of HMSN-SH

The thiolated HMSN (HMSN-SH) was prepared by using PTBA spheres as the templates through a co-condensation process of TEOS and 3-mercaptopropyltrimethoxysilane. Poly (*tert*-butylacrylate) (PTBA) spheres were synthesized according to our previous work.[31] PTBA spheres (0.62 g, 10.8 wt % solid content) were dispersed in a mixture of ethanol (20 mL) and deionized water (50 mL). Then, 10 mL of deionized water containing 0.3 g of CTAB was added into above solution. After stirred vigorously at 40 ^oC for 30 min, 0.85 mL of ammonia solution

(25 wt %), 1 mL of TEOS and 0.2 mL of 3-mercaptopropyltrimethoxysilane were added successively to the mixture. After reaction for 24 h, the produced particles were isolated by centrifugation and washed with ethanol for three times. The obtained particles were then refluxed in ethanol containing 10 mg mL^{-1} of NH_4NO_3 at 80 ^oC for 6 h to remove the templates. After washing with ethanol three times, HMSN-SH was obtained and kept in ethanol.

Synthesis of HMSN-*s*-*s*-Py

200 mg of HMSN-SH was first dispersed in 20 mL of ethanol. Then, 12 μL of acetic acid and 0.11 g of 2,2'-dithiodipryidine were added into the suspension. After reaction for 24 h, HMSN-*s*-*s*-Py was collected by centrifugation and washed with ethanol and deionized water for three times, respectively.

Synthesis of Tf-SH

Tf was modified with sulfydryl groups to obtain thiolated Tf (Tf-SH). A solution containing HEPES (30 mM) and EDTA (1 mM) with a pH value of 8.5 was prepared, denoted as HEB. 10 mg of Tf was added in 1 mL HEB solution and stirred for 20 min under dynamic nitrogen atmosphere. After that, 0.1 mL of ITH (6.5 mM in HEB) was added and kept the mixture at room temperature for 3 h to obtain Tf-SH.

Synthesis of DOX-HMSN-*s*-*s*-Tf

10 mg of HMSN-*s*-*s*-Py was dispersed in 3 mL of DOX solution (1mg mL^{-1}), which was previously adjusted to pH 8.0. After stirring for 24 h, DOX-HMSN-*s*-*s*-Py was obtained by centrifugation and washed with deionized water for twice. The above prepared Tf-SH solution was then added in to a 10 mL solution containing the DOX-HMSN-*s*-*s*-Py and 6 μL of acetic acid and stirred for 24 h. Finally, DOX-HMSN-*s*-*s*-Tf was collected by centrifugation and washed with a pH 5.5 buffer twice to remove the obtained pyridine-2-thione. The amount of DOX in the nanocarriers was calculated by subtracting the mass of DOX in the collected supernatants from all the above procedures from the total mass of drug initial solution by UV-vis at 480 nm using a pre-established calibration curve.

In vitro DOX release experiments

DOX-HMSN-*s*-*s*-Tf (2 mg) was dispersed in 2 mL of PBS (10 mM NaCl, pH 6.5), and the dispersion was placed into a dialysis bag with molecular weight cut-off of 14000 Da. Then, the dialysis bag was transferred into a 150 mL light-sealed sink containing 100 mL of PBS (10 mM NaCl, pH 6.5). At certain time intervals, a portion of the release medium (3 mL) was taken out to measure the released drug concentration and then same volume of fresh PBS solution was added into the sink. After 4 h, GSH was added into the sink with different concentrations (0, 1 and 10 mM).

Quantitative analysis by flow cytometry

A549 cells (human adenocarcinomic alveolar basal epithelial cell line) and 293T cells (human Embryonic Kidney, normal cell) were cultured in 10 cm tissue culture dishes at a density of 1×10^5 cells mL^{-1}. The cells were incubated with DOX-HMSN-SH and DOX-HMSN-*s*-*s*-Tf (50 μg mL^{-1}) for 2 and 4 h in a humidified atmosphere with 5% CO_2 at 37 ^oC, respectively. To investigate the uptake mechanism, A549 cells were treated with pure Tf at different concentrations (0, 0.5 and 1 mg mL^{-1}). Then, the cells were incubated with DOX-HMSN-*s*-*s*-Tf (50 μg mL^{-1}) for 4 h in a humidified atmosphere with 5% CO_2 at 37 ^oC. Finally, the cells were washed with PBS (pH 7.4) for three times and collected by trypsinization. The uptake efficiency of the nanocarriers was measured by flow cytometry.

Cytotoxicity assay by MTT

Free DOX, DOX-HMSN-SH and DOX-HMSN-*s*-*s*-Tf were incubated with either A549 cells or 293T cells for 24 h at different concentrations. Then, the medium was removed, and MTT (0.5 mg mL^{-1}) in DMEM was added into each well. The cells were cultured for 4 h in the dark. Finally, the supernatant was removed and dimethyl sulfoxide was added into the well. The optical density value was measured by a microplate reader at 570 nm.

In vivo tumor-targeting investigation

Male Balb/c mice, weighing 20-25 g and aging 4-5 weeks, were provided from Department of Experimental Animals, Fudan University (Shanghai, China). All animal experiments were performed according to the guidelines evaluated and got approval from Ethics Committee of Fudan University. A549 cells (2×10^6 cells mL^{-1} in PBS) were injected subcutaneously into the flank region, and 3 weeks after that the mice were used for *in vivo* experiments.

To monitor the nanoparticles *in vivo*, indocyanine green (ICG), a near-infrared dye, was loaded in the nanoparticles instead of DOX, denoted as ICG-HMSN-*s*-*s*-Tf. The mice were intraperitoneally injected with ICG-HMSN-*s*-*s*-Tf at a dosage of 20 mg kg^{-1}. In control groups, ICG-HMSN-SH and ICG were also intraperitoneally injected. Then, at 1, 12, 24 and 48 h post injection, NIRF imaging was performed using In Vivo Xtreme. At 1 and 48 h post injection, the major organs and the tumor tissues were collected and used for *ex vivo* NIRF imaging. All the fluorescence images were operated with the same imaging parameters (Excitation: 720 nm; Emission: 790 nm; Exposure time: 10 s).

Evaluation of therapeutic efficacy *in vivo*

24 mice beard with A549 tumor were divided into four groups, which were intraperitoneally injected with saline, free DOX, DOX-HMSN-SH and DOX-HMSN-*s*-*s*-Tf, respectively, with a DOX dosage of 5 mg kg^{-1} per mouse every 2 days and lasted for 8 days. After the first injection, the tumor size and mice weight were measured once every 2 days. The tumor sizes were measured by a caliper and the tumor volume was calculated as (tumor length) × $(\text{tumor width})^2/2$. Relative tumor volumes were calculated as V/V_0 (V_0) was the tumor volume when the treatment was initiated. After treatment for 14 days, the tumor tissues were taken out from the mice for the evaluation of the therapeutic efficacy.

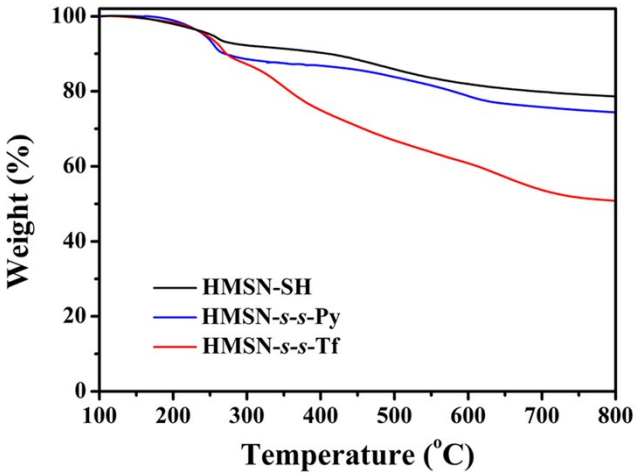

Fig. S1 TGA curves of HMSN-SH, HMSN-*s*-*s*-Py and HMSN-*s*-*s*-Tf.

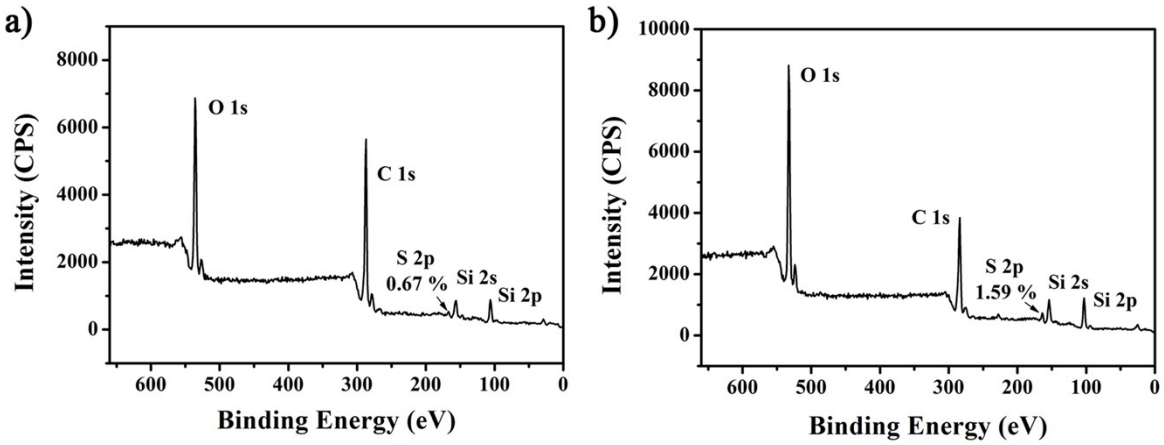

Fig. S2 XPS spectra of (a) HMSN-SH and (b) HMSN-*s*-*s*-Py. After treatment with 2,2'-dipyridyl disulfide, the content of element S on the surface increases from 0.67 % to 1.59 %.

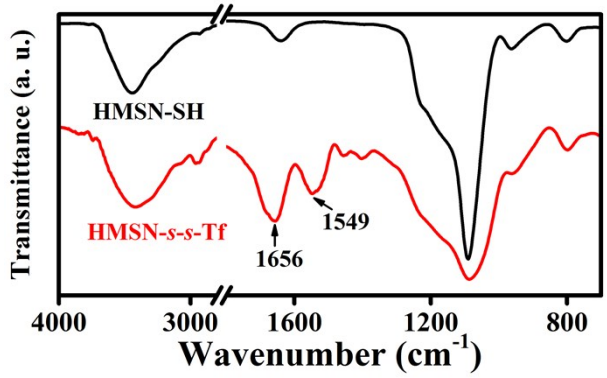

Fig. S3 FTIR spectra of (a) HMSN-SH and (b) HMSN-*s*-*s*-Tf. The bands appearing at 1656 and 1549 cm^{-1} are attributed to the typical amide I and II bands of Tf protein, demonstrating that Tf has been successfully introduced.

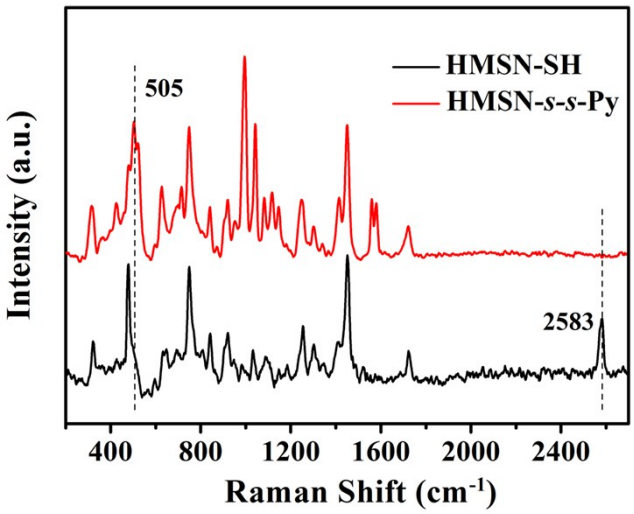

Fig. S4 Raman spectra of HMSN-SH and HMSN-*s*-*s*-Py. The peaks at 2583 and 505 cm^{-1} represent the characteristic peaks of thiol group and disulfide bond, respectively. The absence of the peak at 2583 cm^{-1} and the presence of the peak at 505 cm^{-1} confirm the successful transition of the thiol group to disulfide bond on the particle surface.

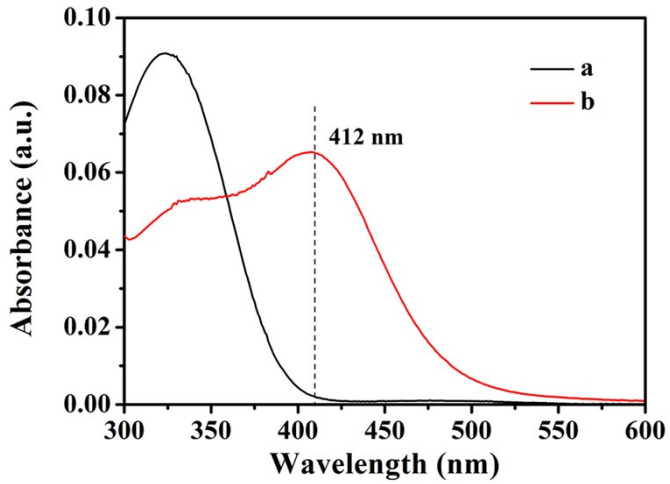

Fig. S5 UV-vis spectra of the DTNB solution (3 mL, 5 μM, pH 8.5) before (a) and after (b) addition of 20 μL of Tf-SH solution. The new peak at 412 nm, corresponding to the characteristic absorption of the reaction product 5-thiol-2-nitrobenzoic acid (TNB^{2-}) of DTNB and thiol group, indicates the successful modification of Tf with thiol group.

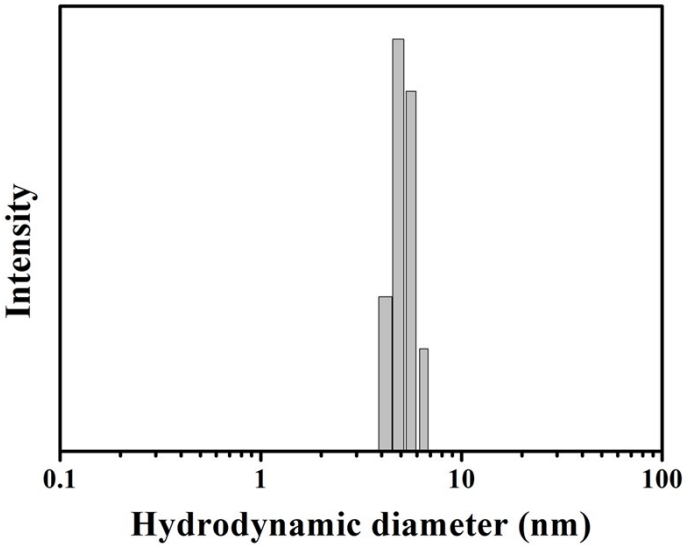

Fig. S6 Hydrodynamic diameter distribution of Tf-SH in PBS (pH 7.4).

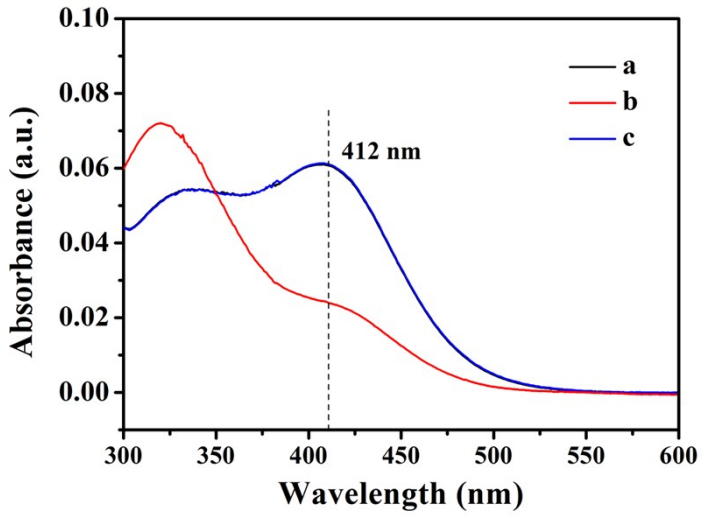

Fig. S7 UV-vis spectra of the DTNB solution (3 mL, 5 μM, pH 8.5) after treatment with (a) Tf-SH solution, (b) the supernatant (400 μL) after the reaction of Tf-SH and HMSN-*s*-*s*-Py, and (c) the supernatant (400 μL) after the reaction of Tf-SH and HMSN-SH. Compared with the absorbance of Tf-SH at 412 nm, the decrease of spectrum b indicates that part of Tf-SH is modified on the surface of HMSN-*s*-*s*-Py to form HMSN-*s*-*s*-Tf. There is no change between spectrum a and c, indicating that Tf could not be physically adsorbed on the surface of HMSNs.

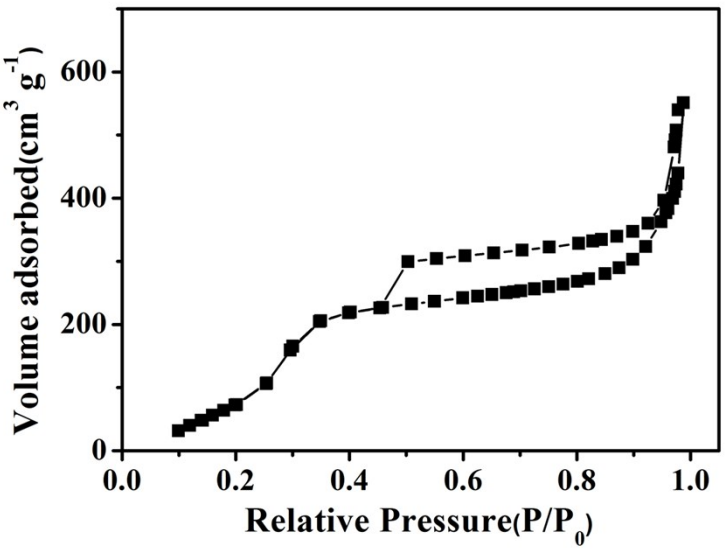

Fig. S8 The N_2 adsorption-desorption isotherm of DOX-HMSN-*s*-*s*-Py. Similar to the HMSN-*s*-*s*-Py nanocarriers, DOX-loaded HMSN-*s*-*s*-Py still has a type IV isotherm and a high surface area of 680 m^2 g^{-1}, suggesting DOX was mainly loaded in the hollow cores not the mesoporous shells.

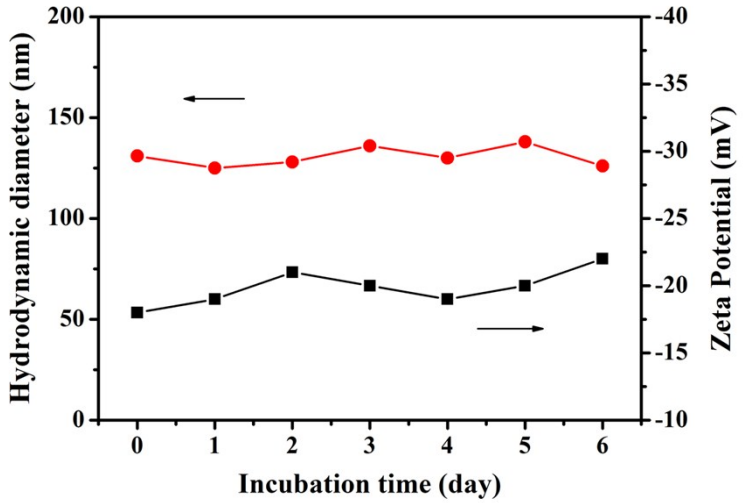

Fig. S9 The real-time measurement of hydrodynamic diameter (left) and zeta potential (right) of DOX-HMSN-*s*-*s*-Tf in PBS buffer (pH 7.4).

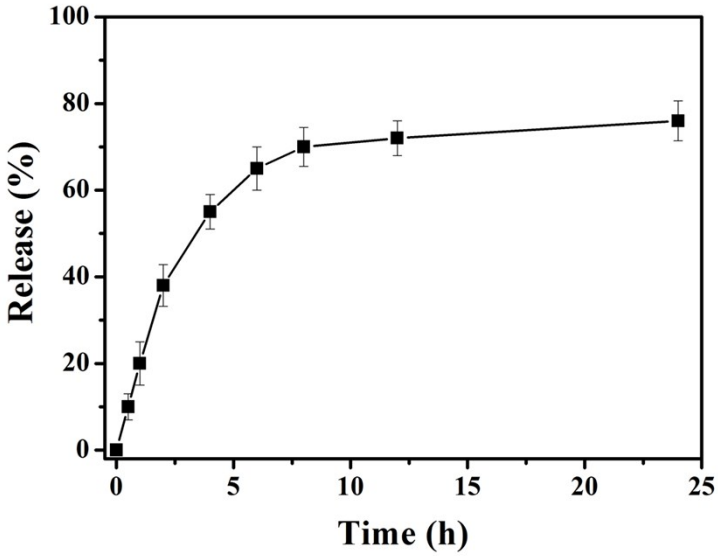

Fig. S10 The release profile of DOX from DOX-HMSN-SH in PBS (pH 6.5).

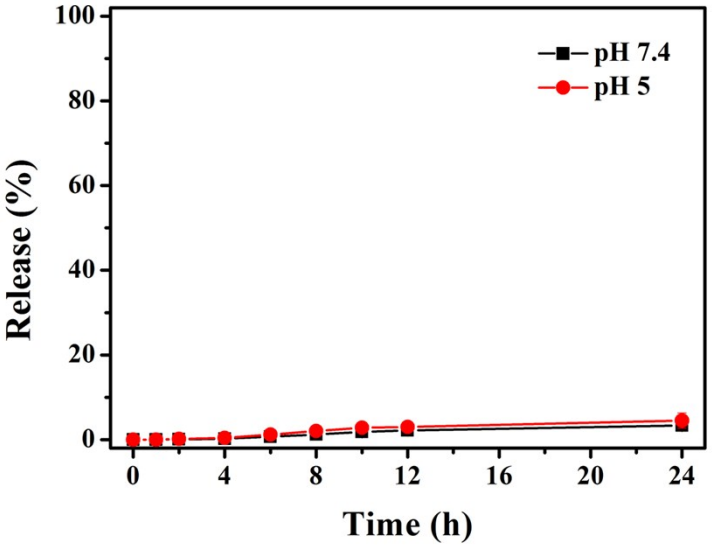

Fig. S11 The release profiles of DOX from DOX-HMSN-*s*-*s*-Tf in PBS at different pH values.

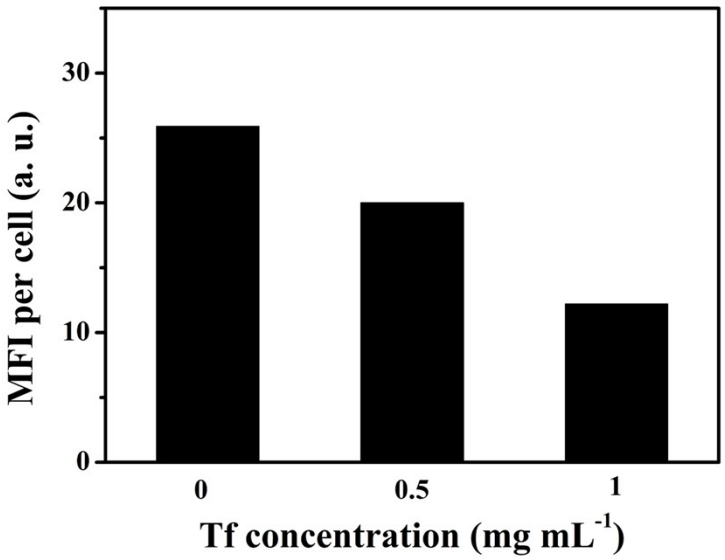

Fig. S12 MFI per cell of A549 cells treated with Tf at different concentrations after incubation with DOX-HMSN-*s*-*s*-Tf for 4 h.

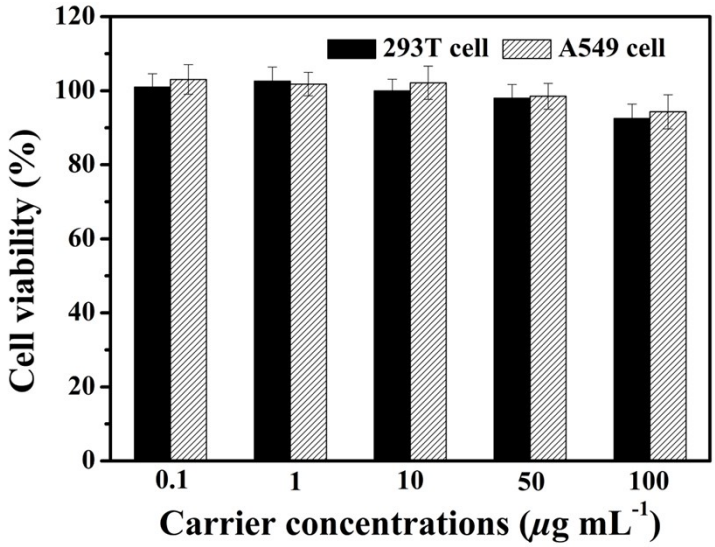

Fig. S13 The cytotoxicity of HMSN-*s*-*s*-Tf to A549 cell and 293T cell in 24 h.

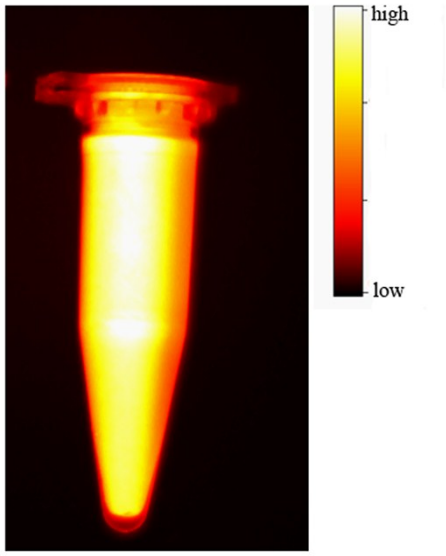

Fig. S14 *In vitro* NIRF image of ICG-HMSN-*s*-*s*-Tf.

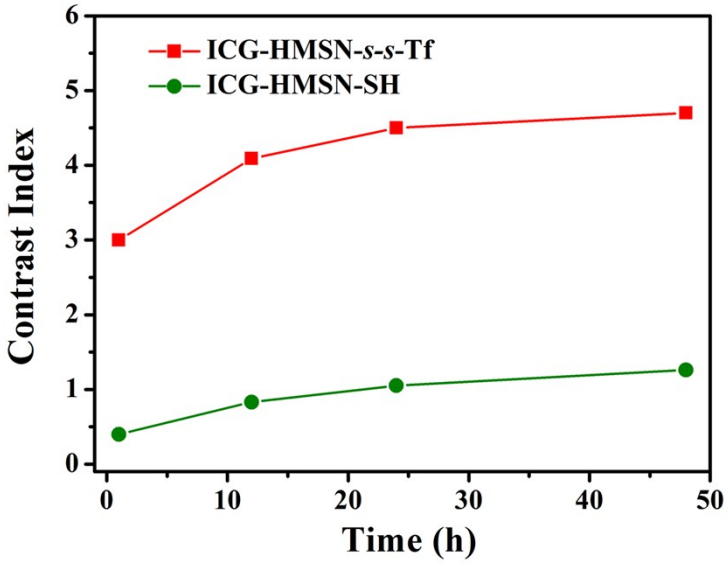

Fig. S15 The contrast index (tumor/normal tissues) of the mice after injection with ICG-HMSN-*s*-*s*-Tf or ICG-HMSN-SH.

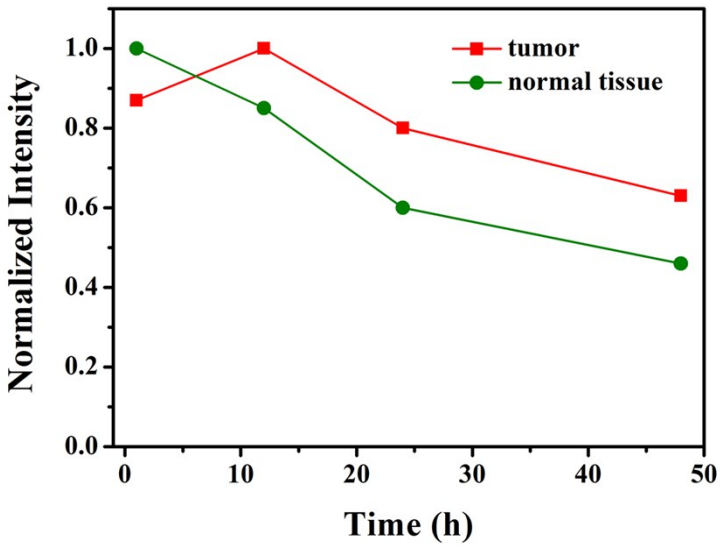

Fig. S16 Accumulation and retention kinetics of ICG-HMSN-*s*-*s*-Tf in tumor and normal tissues.