Electronic Supplementary Information

of

A Redox-Responsive Mesoporous Silica Nanoparticle Capped with Amphiphilic Peptieds by Self-assembly for Cancer Targeting Drug Delivery

Dong Xiao, Hui-Zhen Jia, Ning Ma, Ren-Xi Zhuo, and Xian-Zheng Zhang*

Key Laboratory of Biomedical Polymers of Ministry of Education & Department of Chemistry, Wuhan University, Wuhan 430072, China

* To whom correspondence should be addressed. Tel. & Fax: 86-27-68754509.

E-mail address: xz-zhang@whu.edu.cn (X. Z. Zhang).

1. Materials

Hexadecyl trimethyl ammonium bromide (CTAB), tetraethylorthosilicate (TEOS), sodium hydroxide (NaOH), dichloromethane (DCM), methanol, hydrofluoric acid (HF), piperdine, triisopropylsilane (TIS), were obtained by Shanghai Reagent Chemical Co. (China) and used directly. Stearic acid (C_{18}) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Chain) and used directly. DL-Dithiothreitol (DTT) was purchased from Regal Biotechnology Co. (China) and used directly. Diisopropylethylamine (DIEA), dimethylformamide (DMF), trifluoroacetic acid (TFA), were obtained by Shanghai Reagent Chemical Co. (China) and used after distillation. N-fluorenyl-9-methoxycarbonyl (Fmoc) protected L-amino acids: Fmoc-Gly-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Cys(Trt)-OH, 1-hydroxybenzotriazole (HOBt), o-benzotriazole-N,N,N′,N′,-tertamethyluroniumhexafluorophosphate (HBTU), 2-cholrotriyl chloride resin (100-200 mesh, loading: 1.41 mmol/g) were purchased from GL Biochem Ltd. (Shanghai China) and used as received. Doxorubicin (DOX) was purchased from Zhejiang Hisun Pharmaceutical Co. (China). 3-Mercaptopropyltrimethoxysilane, 2-2′-dithiodipyridine were purchased from Aladdin-reagent.

U-87 MG cells and COS7 cells were purchased from Cell Bank of Chinese Academy of Sciences (Shanghai,China). Fetal bovine serum (FBS), Dulbecco's Modified Eagle's Medium (DMEM), Hoechst 33258 and Dulbecco's phosphate-buffered saline (PBS), penicillin–streptomycin, trypsin, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Invitrogen Corp. Other reagents were analytical grade and used as received.

2. Synthesis of amphiphilic peptide C_{18}-DSDSDSDSRGDS, control group C_{18}-DSDSDSDSDSDS and alkyl chain with thiol group C_{18}-Cys

The amphiphilic peptide C_{18}-DSDSDSDSRGDS was synthesized manually using standard fluorenylmethoxycarbonyl (Fmoc) solid phase peptide synthesis (SPPS) technique.[S1] Briefly, the peptides were grown on a 2-chlorotrityl chloride resin (1.41 mmol/g) in a stepwise way. The coupling of the first residue used 4 eqiv (relative to the substitution degree of degree) of Fmoc-protected amino acid and 6 equiv of DIEA in a DMF solution for 2 h. Other amino acid couplings were carried out with 4 equiv of Fmoc-protecting amino acid, 4 equiv of HBTU, HOBT, and 6 equiv of DIEA for 4 h. During the synthesis process, the Fmoc protecting groups were deprotected with 20% (v/v) piperidine/DMF twice and 20 min per time. At the end of the synthesis, stearic acid was conjugated to the peptide segments by stirring overnight with mixed solution containing stearic acid, HBTU, HOBT and DIEA. After the completion of the synthesis, the resin was finally washed with DMF (four times), methanol (four times) and DCM (four times) and dried under vacuum for 24 h. Cleavage of the expected peptide and the removal of side chain protected groups from the dried resin were performed by suspending the resin in a cleavage cocktail containing TFA, TIS, and H_2O in the volume ratio of 95 : 2.5 : 2.5 for 2 h. The filtrate was concentrated and precipitated in cold ether to obtain the rough product. The product was collected and vacuum dried, then dissolved in distilled water and freeze-dried. The control group C_{18}-DSDSDSDSDSDS and alkyl chain with thiol group C_{18}-Cys were synthesized as described above. The molecular weights were determined by electrospray ionization mass spectrometry (ESI-MS) system (Finnigan LCQadvantage).

3. Synthesis of MSN

MCM-41 type mesoporous silica nanoparticle (MSN) with an average diameter of 125 nm was synthesized according to the base-catalyzed sol-gel method as reporetd in

the literature.[S2] Briefly, 1.0 g CTAB and 280 mg NaOH were dissolved in 480 mL of distilled water. After stirring at room temperature for 20 min, 5.0 g TEOS was added dropwise to the solution. The reaction mixture was vigorously stirred at 80 ^{o}C for another 2 h. The resulting solid was filtered, washed thoroughly with water and methanol and dried under vacuum overnight. The obtained MSNs were characterized by transmission electron microscopy (TEM, JEOL-2100, Japan), scanning electron microscopy (SEM, FEI-QUANTA 200) and Brunauer-Emmett-Teller (BET) and Barrett–Joyner–Halenda (BJH) analysis (ASAP 2020, Micromeritics).

4. Synthesis of MSN-SH

The surface of MSN (880 mg) in methanol (70.5 mL) was functionalized with 2 mL of 3-mercaptopropyltrimethoxysilane. The mixture was stirred at room temperature overnight. The particles were separated by centrifugation (8000 r/min, 10 min), washed six times with methanol and dried under vacuum. The CTAB surfactants were removed by heating the mixture in 76.6 mL methanol and 4.68 mL HCl (37.4 %) at 80 ^{o}C for 48 h. The surfactant removed MSN-SH nanoparticles were purified by centrifugation (8000 r/min, 10min), washed thoroughly with water and then methanol for six times and dried under vacuum.

5. Synthesis of MSN-S-S-Pyridine

The surface of MSN-SH (100 mg) in methanol (25 mL) was modified with 600 mg 2-2′-dithiodipyridine. The mixture was stirred at room temperature in the dark for 24 h. The resulting particles were gained by centrifugation (8000 r/min, 10 min), washed six times with methanol and dried under vacuum.

6. Drug (DOX) loading and synthesis of MSN-S-S-C_{18}/DOX

100 mg MSN-S-S-Pyridine was dissolved in 8 mL DMF and 30 mg DOX was added to the solution under vigorous stirring. After stirring at room temperature in the

dark overnight, 100 mg alkyl chain with thiol group (C_{18}-Cys) was added. The mixture reacted at room temperature in the dark for 24 h. The resulting rough product was centrifuged (8000 r/min, 10 min), washed thoroughly with water only once, and then dried under vacuum.

7. Synthesis of MSN-S-S-C_{18}-Peptide/DOX (RRMSN/DOX), control group MSN-S-S-C_{18}-Peptide/DOX (CRRMSN/DOX) and DOX unloaded MSN-S-S-C_{18}-Peptide (RRMSN)

200 mg amphiphilic peptied (AP) C_{18}-DSDSDSDSRGDS was sonicated in 12 mL deionized water, and 100 mg MSN-S-S-C_{18}/DOX was slowly added to the system. The mixture were stirred at room temperature in the dark for 24 h. The resulting solid was centrifuged (8000 r/min, 10 min), washed thoroughly with DOX solution, water and methanol, and then dried under vacuum. Thereupon, the RRMSN/DOX was fabricated. Control group MSN-S-S-C_{18}-Peptide/DOX (CRRMSN/DOX) and DOX unloaded MSN-S-S-C_{18}-Peptide (RRMSN) were synthesized as detailed above.

8. Measurement of drug loading efficiency and drug encapsulation efficiency

To determine the drug loading level of the redox-responsive drug delivery, 2.0 mg RRMSN/DOX was dispersed in 600 μL HF and then diluted with 9.4 mL H_2O. The mixture was analyzed by RF-5301PC spectrofluorophotometer (Shimadzu). The emission and excitation slit widths were set at 5 nm with λ_{ex} 488 nm (doxorubicin), using a fluoescence rstandard calibration curve. The drug loading efficiency (DLE) was defined as follow: DLE = (mass of drug loaded in MSNs/mass of drug loaded MSNs) × 100%. The drug encapsulation efficiency (DEE) was difined as follow: DEE= (mass of drug loaded in MSNs/mass of feed drug) × 100%. We also measured the DLE and DEE by ultraviolet (UV) absorbance (PerkinElmer UV Spectrometer Lambda 35) intensity at 480 nm, using a ultraviolet standard calibration cruve.

9. *In Vitro* Release Experiments

The closing and opening of the drug delivery nanoparticle gate were investigated by studying the release profiles of RRMSN/DOX.

1) The release profiles of DOX with and without DL-dithiothreitol (DTT): 2.0 mg of MSN-S-S-C_{18}-Peptide/DOX (RRMSN/DOX) was dispersed in 4.0 mL phosphate buffer (PBS, pH 7.4), and was divided into two equal parts. 2 mL 10 mmol/L DL-dithiothreitol (DTT) solution was added to one part, and no treatment was done on another. After particular time intervals, the upper liquid were analyzed by RF-5301PC spectrofluorophotometer (Shimadzu). The emission and excitation slit widths were set at 5 nm with λ_{ex} 488 nm (doxorubicin). The release profiles of DOX with and without glutathione (GSH) were studied in an identical way as described above.

2) The release profiles of DOX at different DTT concentrations: 2.0 mg of MSN-S-S-C_{18}-Peptide/DOX (RRMSN/DOX) were dispersed in 2.0 mL DTT solution at different concentrations of 10 mmol/L、1 mmol/L and absence of DTT solution , respectively, at 37 ^{o}C. After particular time intervals, the upper liquid was analyzed by RF-5301PC spectrofluorophotometer (Shimadzu). The emission and excitation slit widths were set at 5 nm with λ_{ex} 488 nm (doxorubicin). The release profiles of DOX at different GSH concentrations were studied in an identical way as described above.

10. *In Vitro* Cytotoxicity Measurement

The MTT assay was used to determine the cytotoxicity of MSN-S-S-C_{18}-Peptide/DOX (RRMSN/DOX) and control group (CRRMSN/DOX). The U-87 MG cells were seeded at a density of 5.0×10^4 cells/well, and allowed to grow on a 96-well dish with 200 μL DMEM containing 10% FBS. After incubation (37 ^{o}C, 5% CO_2) for 24 h, the original culture media in each well was replaced with 200 μL fresh

medium containing the RRMSN/DOX and CRRMSN/DOX nanoparticles at the indicated concentrations (C_{DOX} : 2.5 μg/mL). Then the medium was replaced with 200 μL of fresh medium and 20 μL of MTT (5 mg/mL in PBS buffer) solution was added and further incubated at 37 ^{o}C for 4 h. Subsequently, the medium was removed and 150 μL DMSO was added. The absorbance was measured at 570 nm using a microplate reader (Bio-Rad, Model 550, USA). The relative cell viability (%) was calculated by ($OD_{570sample}/OD_{570control}$) × 100, where $OD_{570control}$ was obtained in the absence of RRMSN/DOX and $OD_{570sample}$ was obtained in the presence of RRMSN/DOX. The actual value was averaged from four independent experiments. In order to investigate the targeting ability of the redox-responsive drug delivery, as a control, the COS7 cell viability of RRMSN/DOX and CRRMSN/DOX were examined in an identical way as described above. So as to study the biocompatibility of the redox-responsive drug delivery, the U-87 MG and COS7 cell viability of DOX unloaded MSN-S-S-C_{18}-Peptide (RRMSN) were examined in identical way as detailed above.

11. Cellular Uptake

The U-87 MG cells were seeded onto 6-well plates at a density of 1.0×10^5 in 1 mL of DMEM medium and allowed to grow for 24 h. Then 1 mL DMEM containing 62 μg RRMSN/DOX and 48 μg CRRMSN/DOX（C_{DOX} : 2.5 μg/mL） at pH 7.4 were added respectively. After incubated with cells for 4 h at 37 ^{o}C, the medium was removed and cells were washed three times. Thereafter, the cell nuclei were stained with 10 μg/mL Hoechst 33342 in 1 mL DMEM without FBS for 15 min. Then the cells were observed by using Confocal Laser Scanning Microscopy (CLSM) (Nikon C1-si, TE2000, Japan). In order to confirm the targeting and responsive release effects, we select non-cancerous COS7 cells (African Green Monkey SV40-transf'd kidney

fibroblast cell line) as control and the fluorescence confocal microscopic images of RRMSN/DOX and CRRMSN/DOX in COS7 cells were further obtained. The treatment was done in an identical way as described above.

12. Flow Cytometery

The U-87 MG cells were seeded in the 6-well plate with a density of 1.0×10^5 cells/well and incubated at 37 ^oC for 24 h. And then 1 mL RRMSN/DOX and CRRMSN/DOX complexes were added, espectively. After incubated with cells for 4 h, the medium was removed. The cells were rinsed with PBS thoroughly. Subsequently, the cells were collected and resuspended in 500 μL PBS three times. The samples were determined on a Beckman Flow Cytometer (Epics XL). The results were analyzed with Flowjo 7.6 software. As a control, the treatment was done in an identical way as described above in COS7 cells.

13. Transmission electron microscopy (TEM)

The samples were carried out on a JEM-2100 instrument operating at an acceleration voltage of 80 KV.

14. Dynamic light scattering (DLS)

The hydrodynamic diameter of nanoparticles in DI water were analyzed on a Nano-ZS ZEN3600 particle sizer (Malvern Instruments).

15. Brunauer-Emmett-Teller (BET) and Barrett-Joyner-Halenda (BJH)

Surface area was calculated by the Brunauer-Emmett-Teller approach and the pore size distributions were obtained by the Barrett-Joyner-Halenda method (ASAP2020, micromeritics).

16. Fourier Transform-Infrared Spectroscopy (FT-IR)

The samples in KBr pellets were analyzed by a Spectrum Two FT-IR spectrophotometer (Perkin-Elmer).

17. Thermal Gravimetric Analysis (TGA)

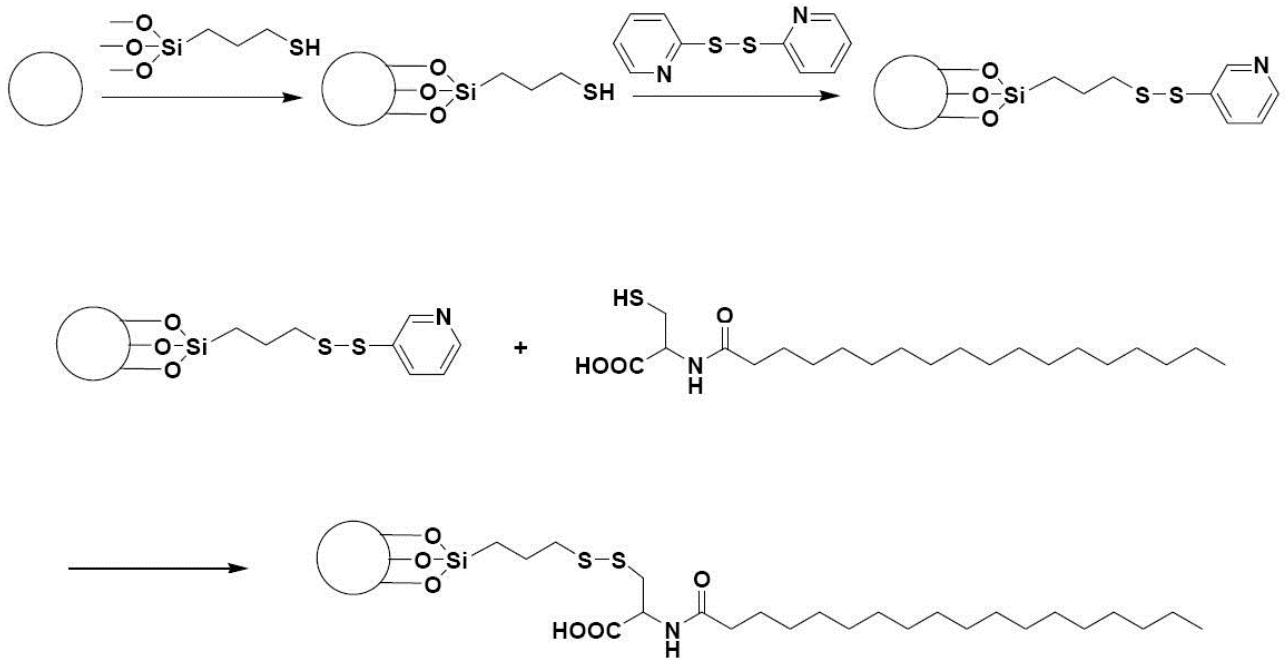
Thermal gravitational analysis (TGA) was performed on a TGS-2 thermogravimetric analyzer (Perkin-Elmer).

Supplementary References

[S1] Z. H. Zhao, D. T. Huang, Z. Y. Yin, X. M. Wang, J. H. Gao, *J. Mater. Chem.* **2012**, *22*, 15717-15725.

[S2] D. R. Radu, C. Y. Lai, K. Jeftinija, E. W. Rowe, S. Jeftinija, V. S.Lin, *J. Am. Chem. Soc.* **2004**, *126*, 13216-13217.

Supplementary Figures



Scheme. S1 Synthesis of the MSN-S-S-C_{18}.

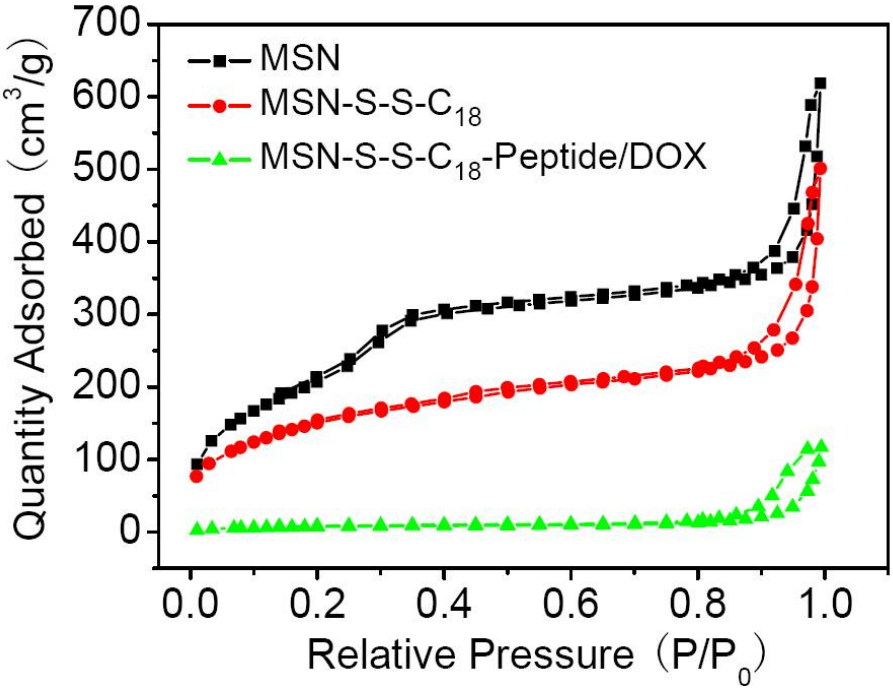


Fig. S1 Brunauer Emmett Teller (BET) of MSN, MSN-S-S-C_{18}, MSN-S-S-C_{18}-Peptide/DOX (RRMSN/DOX).

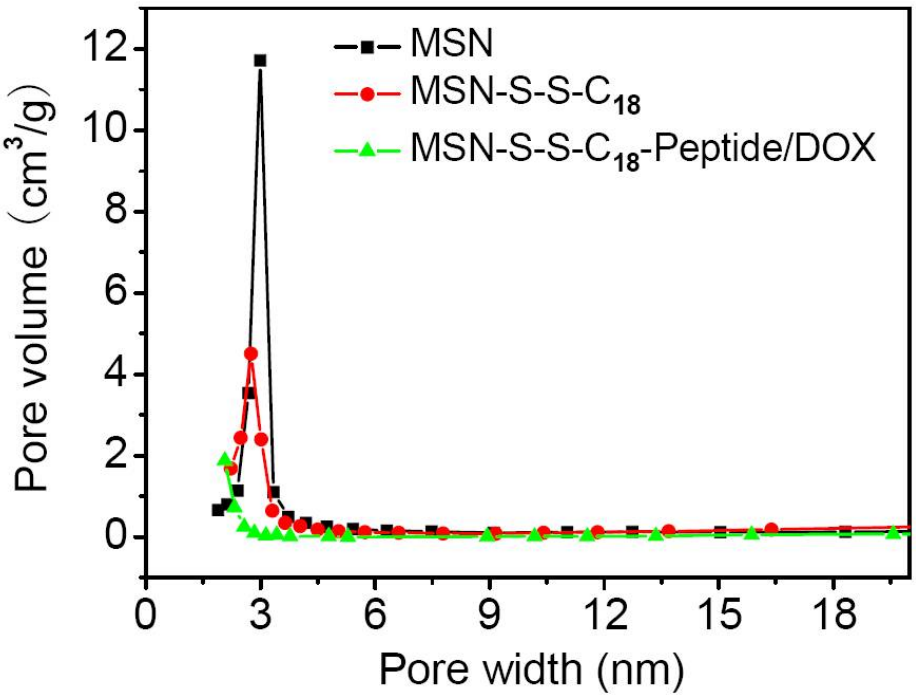


Fig. S2 Barrett-Joyner-Halenda (BJH) of MSN, MSN-S-S-C_{18}, MSN-S-S-C_{18}-Peptide/DOX (RRMSN/DOX).

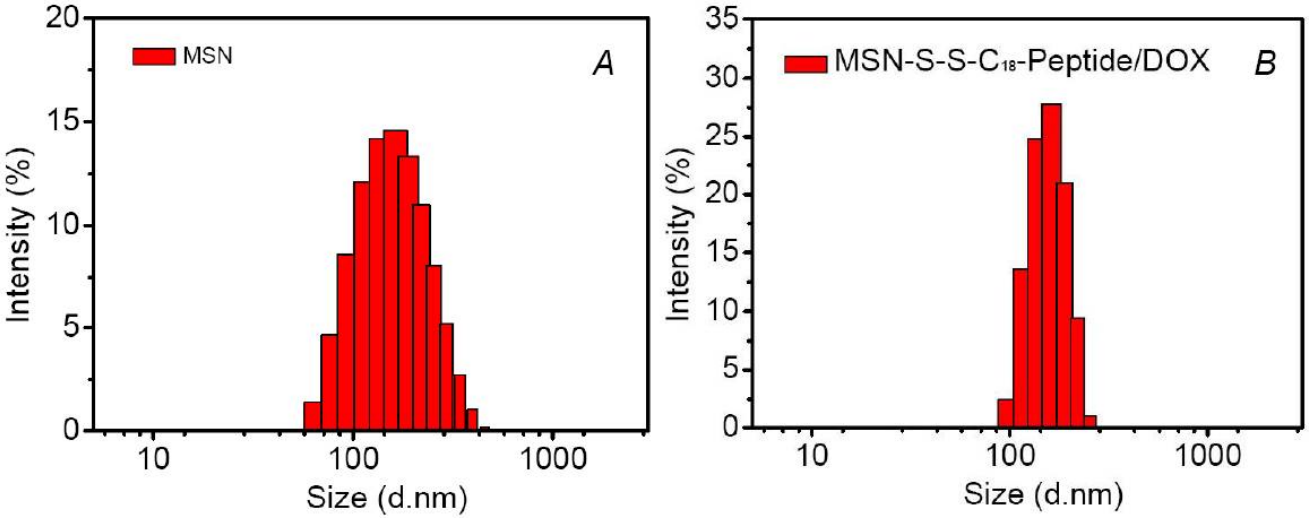


Fig. S3 Particle size distributions detected by dynamic light scattering (DLS) in distilled water. (A) Size distribution of MSN (PDI=0.175); (B) Size distribution of MSN-S-S-C_{18}-Peptide/DOX (RRMSN/DOX) (PDI=0.273).

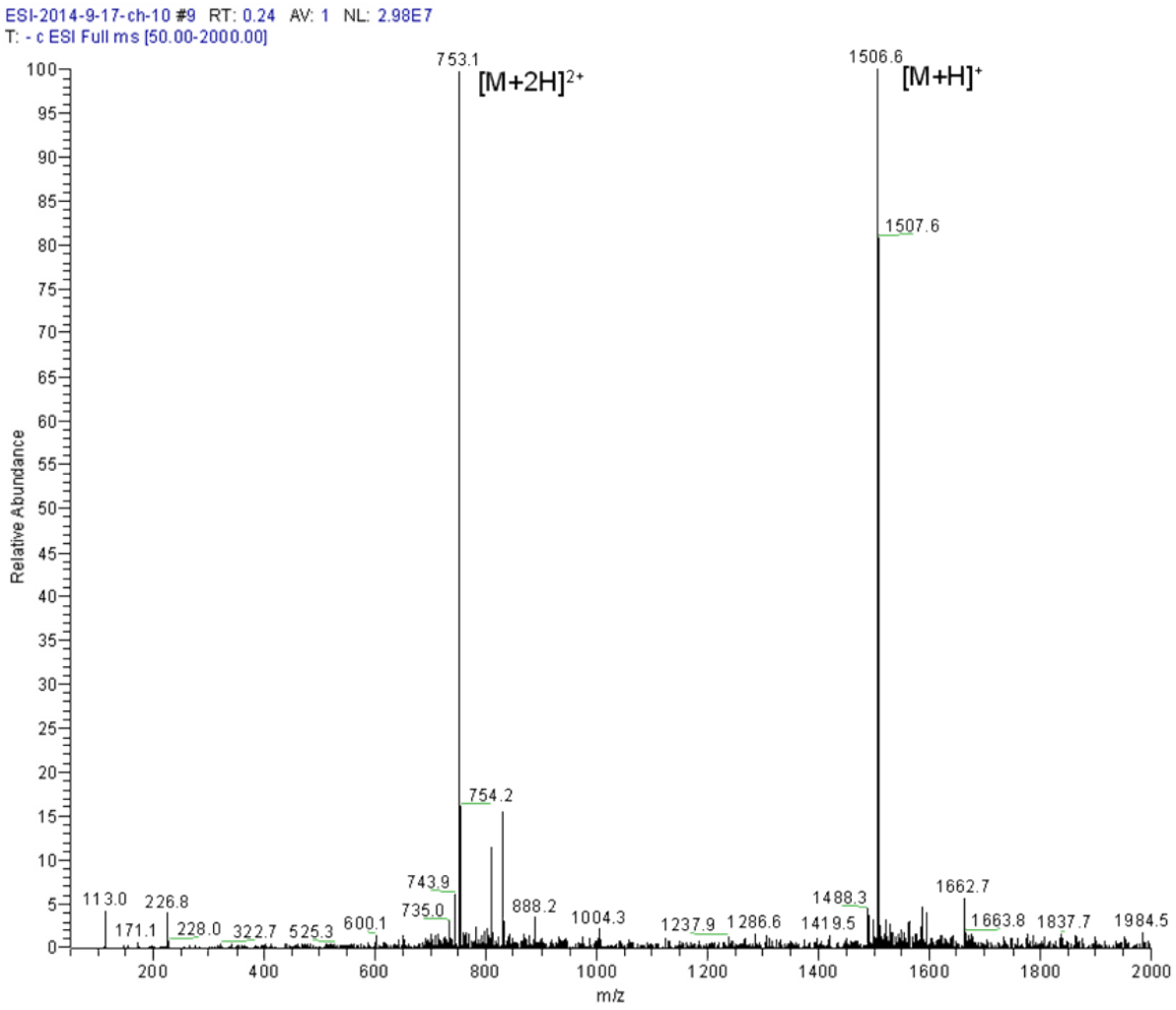


Fig. S4 ESI-MS of amphiphilic peptide C_{18}-DSDSDSDSRGDS

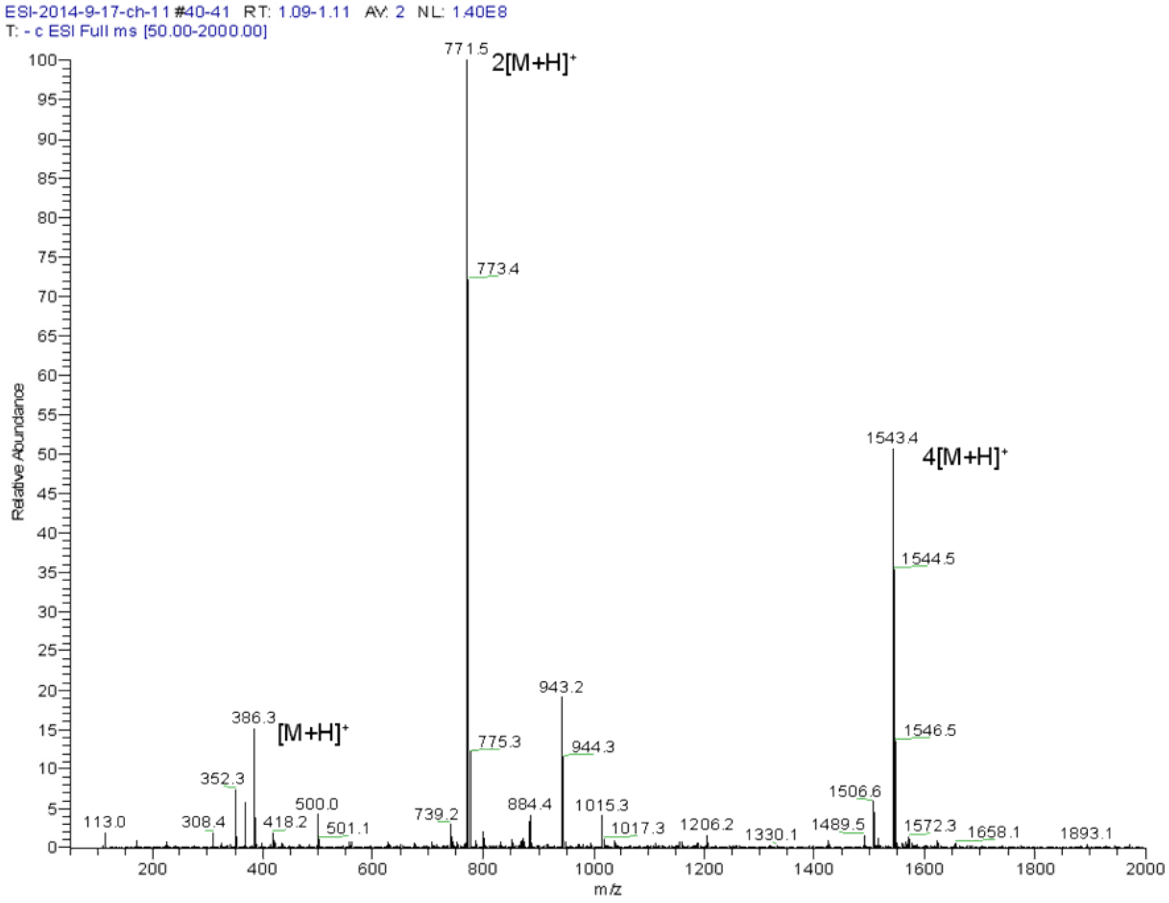


Fig. S5 ESI-MS of alkyl chain with thiol group, C_{18}-Cys

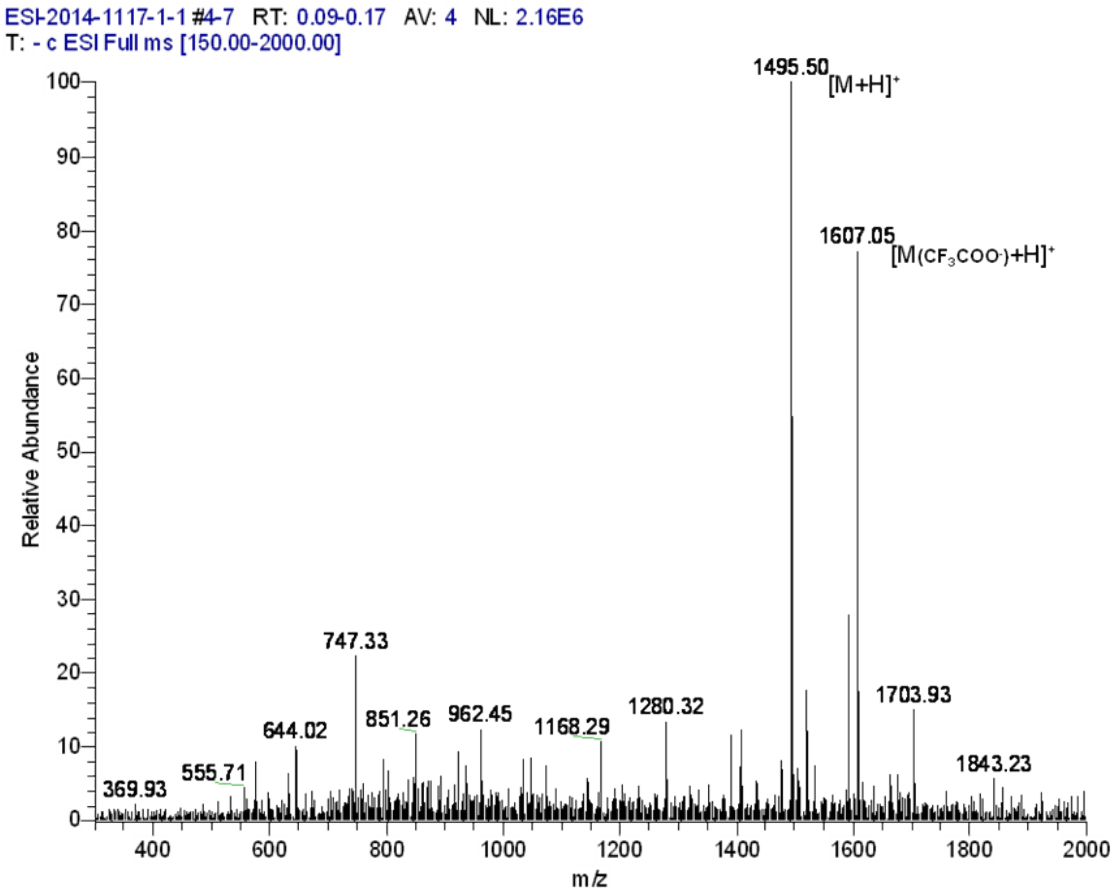


Fig. S6 ESI-MS of control peptide C_{18}-DSDSDSDSDSDS

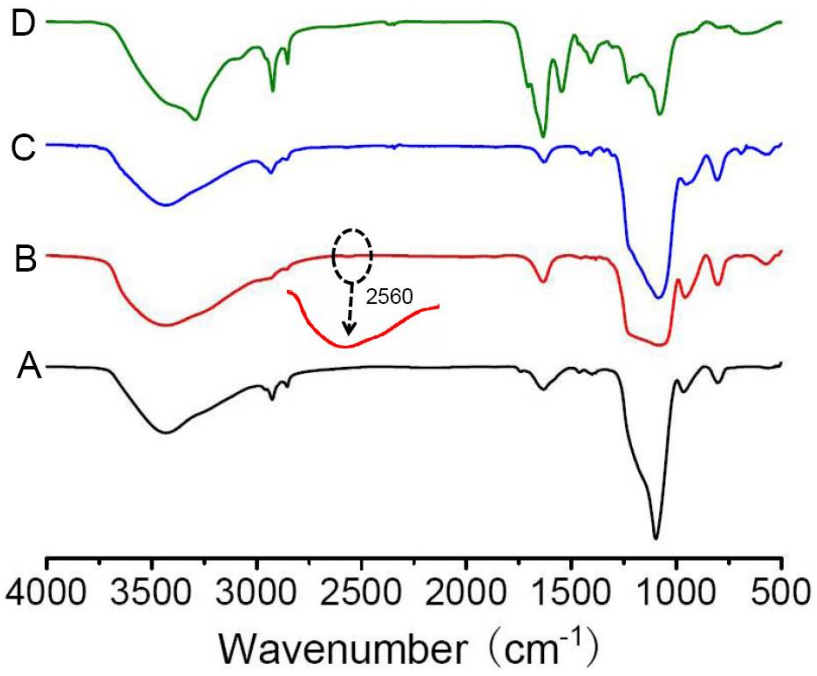


Fig. S7 FT-IR spectra of different nanoparticles. (A) MCM-41; (B) MSN-SH; (C) MSN-S-S-C_{18}; (D) MSN-S-S-C_{18}-Peptide/DOX (RRMSN/DOX).

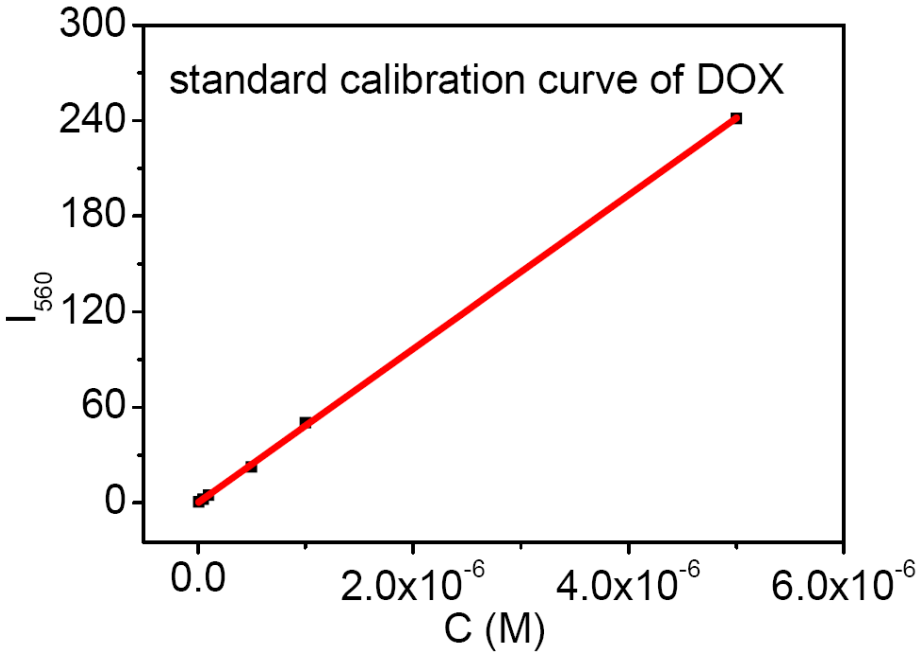


Fig. S8 Fluorescence standard calibration curve of DOX in water.

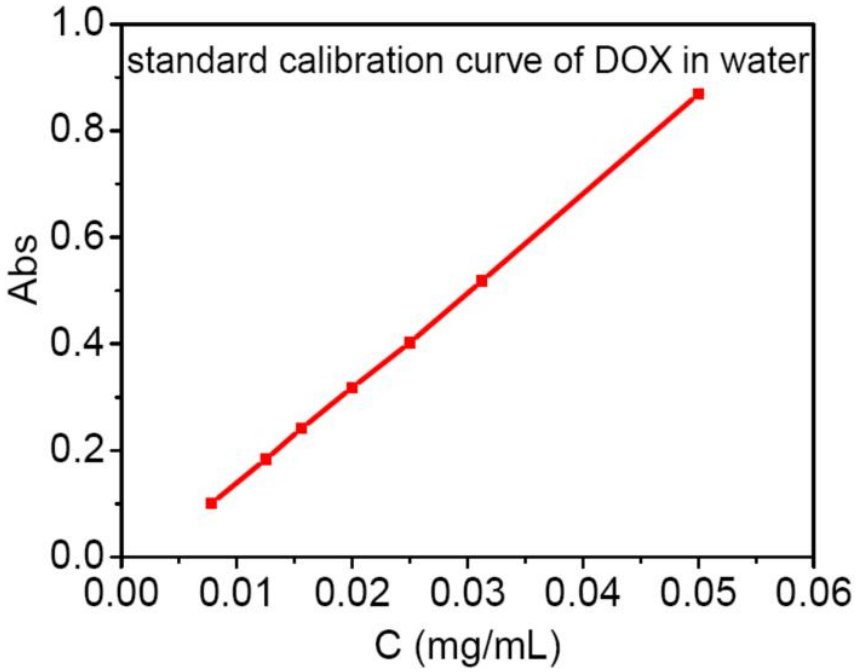


Fig. S9 Ultraviolet standard calibration curve of DOX in water.

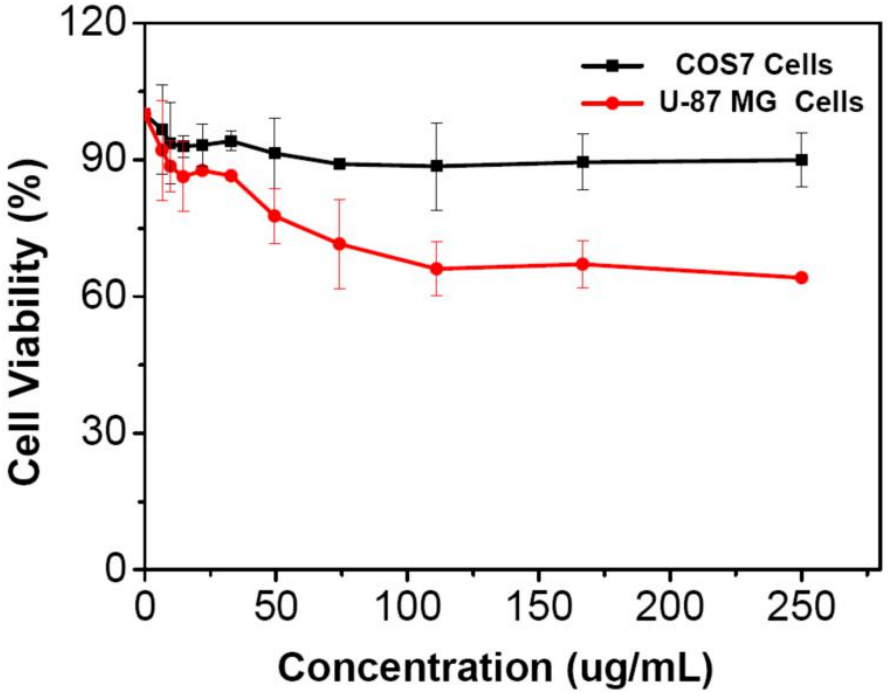


Fig. S10 Viability of U-87 MG cells and COS7 cells after co-incubated with MSN-S-S-C_{18}-Peptide (RRMSN). The concentration of nanoparticle was 250 μg mL^{-1}.

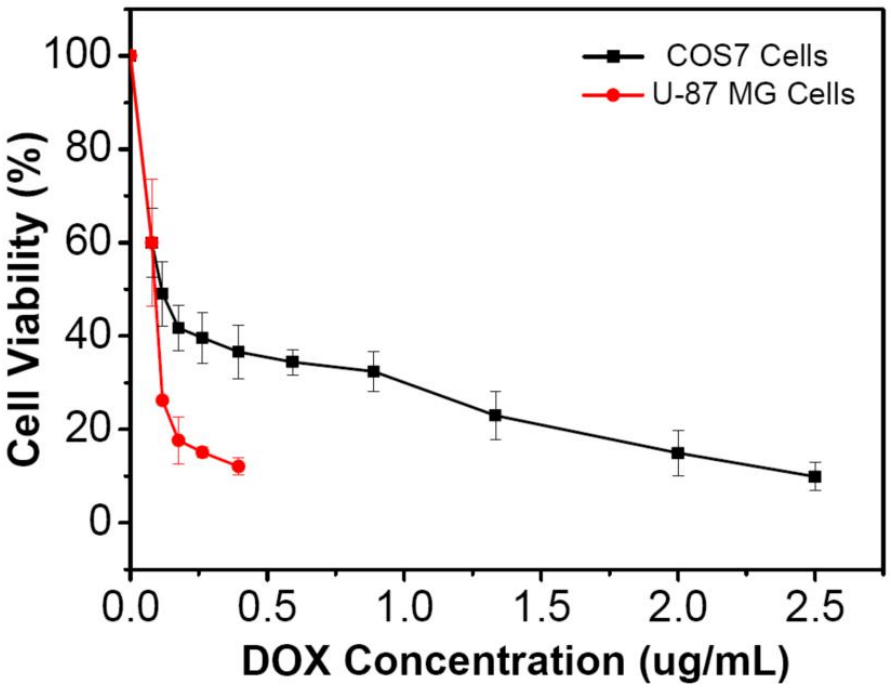


Fig. S11 Viability of U-87 MG cells and COS7 cells after co-incubated with free DOX. The concentration of DOX was 2.5 μg mL^{-1}.