Supporting Information

of

Multifunctional Envelope-Type Mesoporous Silica Nanoparticles for Tumor-Triggered Targeting Drug Delivery

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Experimental section

Materials: Hexadecyl trimethyl ammonium bromide (CTAB), tetraethylorthosilicate (TEOS), $CuSO_4 \cdot 5H_2O$, sodium ascorbate, ethylene diamine tetraacetic acid (EDTA), trifluorocaetic acid (TFA) and tris hydrochloride ultrapure (Tris) were purchased from Shanghai Reagent Chemical Co. (China) and used as received. 3-Mercaptopropyltrimethoxysilane, adamantanecarboxylic acid, propargyl amine, propargyl bromide, 5-aminofluorescein, Brij and 1, 10-phenanthroline monohydrate (MMP inhibitor) were purchased from Aladdin Reagent Co. Ltd. (Shanghai, China). Matrix metalloproteinases (MMP-2) was purchased from RD-SYSTEMS. N-Fluorenyl-9-methoxycarbonyl (Fmoc) protected L-amino acids (Fmoc-Lys-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Val-OH, Fmoc-Arg(pbf)-OH, Fmoc-Asp(otBu)-OH, and H-Asp(obzl)-OH), 2-chlorotrityl chloride resin (2-CTC resin) (100–200 mesh, loading: 1.38 $mmol \cdot g^{-1}$), N, N-diisopropylethylamine (DIEA), o-benzotriazole-N,N,N',N'-tetramethyluronium-hexafluoro-phosphate (HBTU), 1-hydroxybenzotriazole (HOBt), 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium (HATU) and piperidine were purchased from GL Biochem Ltd. (Shanghai, China) and used as received. Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS) and Dulbecco's phosphate buffered saline (PBS) were purchased from Invitrogen Corp. Doxorubicin hydrochloride (DOX) and triisopropylsilane (TIS) were purchased from Zhejiang Hisun Pharmaceutical Co. (China). *S*-(2-Aminoethylthio)-2-thiopyridine hydrochloride, azido-gly and mono-6-azido-β-CD was synthesized according to literature procedures.[S1,12,S2] Diisopropylethylamine (DIEA), dimethylformamide (DMF), tetrahydrofuran (THF) and propargylamine were distilled before use. All other reagents and solvents were provided by Shanghai Reagent Chemical Co. (China) and used without further purification.

Synthesis of MSN: MCM-41 type mesoporous silica nanoparticles (MSN) with an average diameter of 140 nm were synthesized according to a reference procedure.[21] Briefly, CTAB (1.0 g) and NaOH (0.28 g) were dissolved in de-ionized (DI) water (480 mL) and heated up to 80 ^{o}C. Then TEOS (5.0 g) was added dropwise to the solution under vigorous stirring. The reaction mixture was vigorously stirred at 80 ^{o}C for 2 h to obtain MSN. The nanoparticles were then centrifuged (8000 r/min, 10 min), washed thoroughly with water and methanol for several times and dried under vacuum. The obtained nanoparticles were characterized by TEM, scanning electron microscopy (SEM, FEI-QUANTA 200) and Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) analysis (ASAP 2020, Micromeritics).

Synthesis of MSN-SH: MSN (1.35 g) was dispersed in methanol (108 mL). Then 3-mercaptopropyltrimethoxysilane (3 mL) was added and the mixture was stirred for 24 h at room temperature to obtain MSN-SH nanoparticles. The nanoparticles were then centrifuged (8000 r/min, 10 min), washed six times with methanol and dried under vacuum.

Synthesis of MSN-SS-NH_2: MSN-SH (600 mg) was dispersed in methanol (135 mL). Then *S*-(2-aminoethylthio)-2-thiopyridine hydrochloride (600 mg) was added. The mixture was stirred at room temperature for 24 h to obtain MSN-SS-NH_2 nanoparticles. The nanoparticles were then centrifuged (8000 r/min, 10 min), washed six times with methanol and dried under vacuum.

Synthesis of MSN-SS-alkyne: MSN-SS-NH_2 (450 mg) was dispersed in methanol (100 mL) and reaction with propargyl bromide (3 mL) at room temperature for 24 h. The particles was obtained by centrifugation (8000 r/min, 10 min) and washed six times with methanol. CTAB was removed by refluxing with a mixture of methanol (80 mL) and HCl (37.4 %, 4.5 mL) at 60 ^{o}C for 48 h to obtain MSN-SS-alkyne nanoparticles. The nanoparticles were then centrifuged (8000 r/min, 10 min), washed six times with methanol and dried under vacuum.

Synthesis of MSN-SS-CD: MSN-SS-alkyne (100 mg) (with or without 25 mg DOX) was dispersed in a mixture of methanol (5 mL) and PBS (5 mL). After stirring at room temperature for 24 h, mono-6-azido-β-CD (100 mg), $CuSO_4 \cdot 5H_2O$ (100 mg) and sodium ascorbate (170 mg) were added. The mixture was stirred under N_2 atmosphere at room temperature for 3 days to obtain DOX loaded or unloaded MSN-SS-CD nanoparticles. The nanoparticles were then centrifuged (8000 r/min, 10 min), washed thoroughly with EDTA solution, water and methanol, and dried under vacuum.

Synthesis of Fmoc-Lys(AD)-OH: To solution of adamantanecarboxylic acid (3.29 g, 18 mmol) in anhydrous DMF, HATU (6.94 g, 18 mmol), HOBt (2.80 g, 18 mmol) and DIEA (7.077 g, 54 mmol) were added under stirring and the solution was kept in ice-cooled bath and stirred for 10 min. Then Fmoc-Lys-OH (8.07 g, 22 mmol) was added and the suspension was stirred for 48 h at room temperature. The reaction solution was filtrated to remove the unreacted Fmoc-Lys-OH and the solvent was removed in vacuo. The residue was dissolved in 200 mL of EtOAc, washed with $KHSO_4$ (0.5 M, 150 mL×3), water (150 mL×1) and brine (150 mL×1). The organic layer was dried over anhydrous Na_2SO_4, filtrated and concentrated in vacuo. The crude product was purified with column chromatography (EtOAc:hexane 1:1 with 1% HOAc).

Synthesis of N_3GPLGVRGRGDK-Ad and N_3GPLGVRKKK-Ad: N_3GPLGVRGRGDK-Ad and N_3GPLGVRKKK-Ad were synthesized manually by standard Fmoc solid phase peptide synthesis, using 2-chlorotrityl chloride resin (CLTR) and Fmoc-amino acids. The coupling of the first residue used 4 equiv (relative to the substitution degree of resin) Fmoc-protected amino acid (Fmoc-Lys(AD)-OH) 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, and 6 equiv of DIEA in a DMF solution for 4 h. Fmoc protected groups were deprotected with 20% piperidine/DMF (v/v) for twice. At the end of the

synthesis, azido-gly was conjugated to the peptide segments. After the completion of the synthesis, the resin was washed with DMF (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 (95%), TIS (2.5%) and H_2O (2.5%) for 2 h. The filtration was concentrated to a viscous solution by rotary evaporation. After the precipitation in cold ether, the product was collected, vacuum dried, dissolved in distilled water and freeze-dried.

Preparation of MSN-SS-CD-Peptide: MSN-SS-CD-Peptide was prepared through host-guest interaction. MSN-SS-CD (68.5 mg) was dispersed in a mixture of H_2O (7.5 mL) and DMF (2.5 mL). Then N_3GPLGVRGRGDK-Ad (140 mg) was added, and the mixture was stirred for 24 h at room temperature to obtain MSN-SS-CD-Peptide nanoparticles. The nanoparticles were then centrifuged (8000 r/min, 10 min), washed thoroughly with a mixture of water and methanol (V/V=2/1) and dried under vacuum. For comparison, N_3GPLGVRKKK-Ad without RGD sequence was used to prepare MSN-SS-CD-Peptide* nanoparticles under the same conditions.

Synthesis of PASP-alkyne: H-Asp(obzl)-OH (4.4 g, 0.0197 mol) was suspended in dry THF (100 mL) and heated up to 50 ^oC. Then a solution of triphosgene (3.2 g, 0.0107 mol) in dry THF (45 mL) was added dropwise. The mixture was stirred at 50 ^oC under N_2 atmosphere. The reaction was stopped when the suspension became a clear solution. The solution was then poured into excess dried n-hexane to obtain crude product of L-aspartic acid 4-benzyl ester N-carboxyanhydride (Asp(obzl)-NCA). After repeated precipitation with dry THF/n-hexane, the purified product was dried under vacuum.

Asp(obzl)-NCA (4 g, 0.016 mol) and propargyl amine (0.088 g, 0.0016 mol) were dissolved in dry DMF (15 mL) and the mixture was stirred at 50 ^oC for 72 h under N_2 atomosphere. Then the solution was cooled to room temperature and moved into

dialyzing tube (MWCO=1000). PASP(obzl)-alkyne was obtained after dialyzing against DI water and freeze-drying. Subsequently, PASP(obzl)-alkyne (1.83 g) was dissolved in DMF (10 mL). Then NaOH solution (0.5 M, 50 mL) was added slowly into the above solution. The mixture was stirred at room temperature for 24 h and washed with ether (20 mL) for three times. The aqueous phase was combined. After adjusting the pH value to 2 with 0.1 M HCl, the solution was dialyzing against water (MWCO=1000). PASP-alkyne was obtained after lyophilized.

Fluorescent Labeling of PASP-alkyne: PASP-alkyne was fluorescently labeled with 5-aminofluorescein. In brief, PASP-alkyne was dissolved in distilled water and then 5-aminofluorescein (4 wt% based on PASP-alkyne) as well as EDC (1.2-fold molar excess compared with 5-aminofluorescein) was added. After stirring at room temperature for 24 h, 5-aminofluorescein labeled PASP-alkyne was obtained after dialyzing against water and freeze-drying.

Synthesis of MSN-SS-CD-Peptide-PASP (MEMSN): MSN-SS-CD-Peptide (100 mg) was dispersed in DI water (20 mL). Then PASP-alkyne (or labeled PASP-alkyne) (50 mg) was added, followed by the addition of $CuSO_4$ (50 mg) and sodium ascorbate (100 mg). The mixture was stirred at room temperature for 3 days under N_2 atomosphere to obtain MSN-SS-CD-Peptide-PASP (MEMSN). The nanoparticles were then centrifuged (8000 r/min), thoroughly washed with water and dried under vacuum. For comparison, MSN-SS-CD-Peptide*-PASP nanoparticles without RGD sequence were prepared under the same conditions.

In Vitro Drug Release: 3 mg of DOX loaded MSN-SS-CD-Peptide-PASP nanoparticles were suspended in three different release media: phosphate buffer (PBS) with pH 7.4, PBS with 10 mM GSH, and PBS with 1 mM GSH, respectively, at 37 ^{o}C. After particular time intervals, the drug concentration in the release medium was analyzed by RF-5301PC spectrofluorophotometer (Shimadzu). The emission and excitation slit widths were set at 5 nm with λex=470 nm.

MMP Triggered Release of PASP: 20 mg of MSN-SS-CD-Peptide-PASP nanoparticles prepared by labeled PASP-alkyne were dispersed in 3 mL TCNB buffer solution (composed of 100 mM Tris, 5 mM calcium chloride, 200 mM NaCl, and 0.1% Brij) containing MMP-2 (2 μg/mL) (with or without MMP inhibitor) and moved into dialysis tubes (MWCO: 8000-12000 Da). Subsequently, the dialysis tube was immersed in 10 mL TNCB buffer solution and incubated at 37 ^{o}C. The incubation medium was analyzed by Lambda Bio40 UV/Vis spectrometer (Perkin–Elmer) at given time intervals.

Co-Incubation of Nanoparticles with Cells: SCC-7 (squamous cell carcinoma) cells, HT-29 (human colon cancer) cells and 293T (human embryonic kidney 293 transformed) cells were incubated in DMEM medium with 10% FBS and 1% antibiotics (penicillin-streptomycin, 10,000 μ/mL) at 37 ^{o}C in a humidified atmosphere containing 5% CO_2. The cells were seeded in a glass bottom dish and incubated in DMEM (1 mL) containing 10% FBS for 24 h. Thereafter, DOX loaded nanoparticles (125 μg/mL) dispersed in DMEM medium (1 mL) with 10% FBS and 1% antibiotics were added and the cells were further incubated at 37 ^{o}C for another 4 h. After removing the medium and then washing with PBS (1 mL), the cells were observed by using Confocal Laser Scanning Microscopy (CLSM) (Nikon C1-si, BD Laser).

Flow Cytometry. The quantitative evaluation of cellular uptake was performed by flow cytometry (BD FACSAria^{TM} III). SCC-7 cells, HT-29 cells and 293T cells were seeded respectively in 6-well plates (5×10^4 cells/well) and cultured in DMEM (1 mL) containing 10% FBS for 24 h. After that, DOX-loaded nanoparticles (125 μg/mL) dispersed in DMEM medium (1 mL) with 10% FBS and 1% antibiotics were added and the cells were further incubated at 37 ^{o}C for another 4 h. Then the medium was removed and the cells were washed three times with PBS. All the cells were digested by trypsin and collected in centrifuge tubes by centrifugating at 1000 rpm for 5 min. The supernatant was discarded and the bottom cells were washed twice with PBS (pH

7.4). Then the suspended cells were filtrated and examined by flow cytometry. Cells untreated with nanoparticles were used as negative control. The fluorescence scan was performed with 1×10^4 cells.

In Vitro Cytotoxicity: SCC-7 cells, HT-29 cells or 293T cells were seeded into a 96-well plate (1.5×10^4 cells/well) containing DMEM (200 μL). After incubation for 24 h (37 °C, 5% CO_2), half of the culture medium was removed, and DMEM (100 μL) containing a fixed amount of DOX loaded nanoparticles were added in each well. The cells were co-incubated with DOX loaded nanoparticles at 37 °C for 4 h. Then the DMEM medium containing DOX loaded nanoparticles was replaced with 200 μL of fresh DMEM, and the cells were further incubated at 37 °C for 24 h. For comparison, the cell viability of unloaded MEMSN was also investigated. The cells were co-incubated with unloaded MEMSN at 37 °C for 24 h, 48 h, and 72 h, respectively. After the incubation with particular nanoparticles, MTT solution (20 μL, 5 mg/mL) was added to each well and the cells were further incubated for another 4 h. Subsequently, the MTT medium was removed and DMSO (200 μL) was added to each well. The optical density (OD) was measured at 570 nm with amicroplate reader (BIO-RAD 550). The cell viability was calculated as follows: Viability=$(OD_{treated}/OD_{control})\times100\%$, where $OD_{treated}$ was obtained from the cells treated by nanoparticles and $OD_{control}$ was obtained from the cells without any treatments.

^{1}H Nuclear Magnetic Resonance (^{1}H NMR): ^{1}H NMR spectra were recorded on a Mercury VX-300 spectrometer (Varian) at 300 MHz by using $CDCl_3$ or D_2O as the solvent.

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

Zeta Potential Measurement: The zeta potentials of nanoparticles in DI water (pH 7.0) were measured on a Nano-ZS ZEN3600 particle sizer (Malvern Instruments).

Thermal Gravimetric Analysis (TGA): TGA was performed on a TGS-2 thermogravimetric analyzer (Perkin-Elmer).

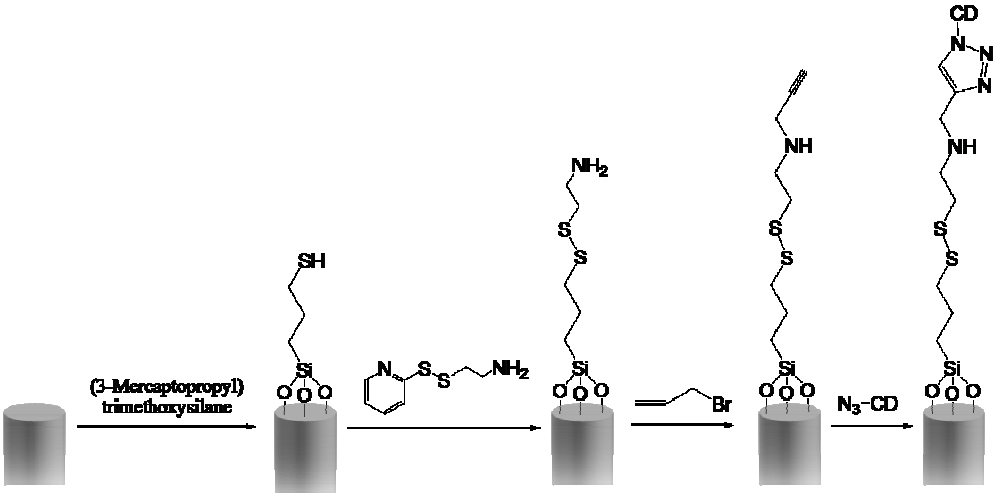
X-ray Diffraction (XRD) Analysis: XRD analysis was performed on a X'Pert Pro diffractometer (PANalytical).

Table S1. Zeta potentials of different nanoparticles in DI water.

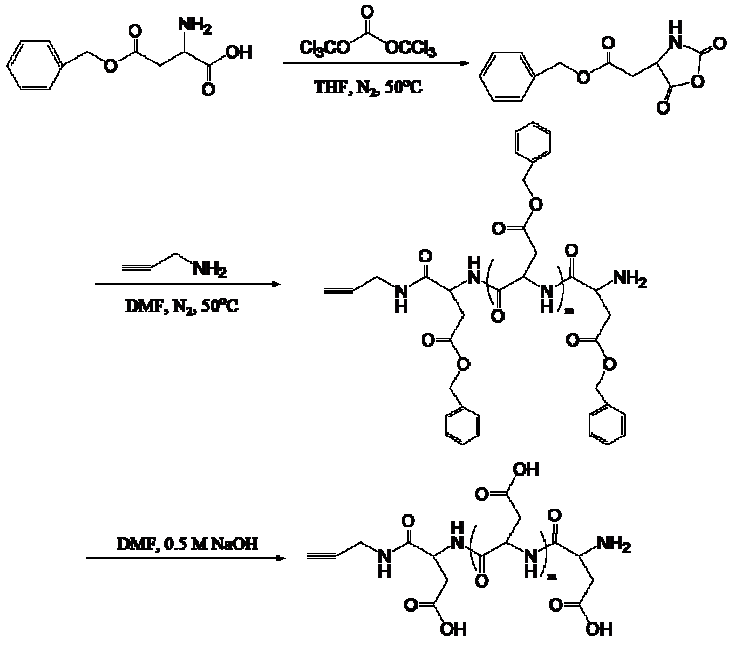
Sample	Zeta potential (mV)
MSN	-24.7
MSN-SH	-18.0
MSN-SS-NH_2	14.7
MSN-SS-alkyne	0.0485
DOX loaded MSN-SS-CD	-12.0
DOX loaded MSN-SS-CD-Peptide	-13.4
DOX loaded MSN-SS-CD-Peptide-PASP	-25.8

Table S2. BET and BJH parameters of different nanoparticles.

Sample	BET surface area S_{BET} (m^2/g)	BET pore volume V_p (cm^3/g)	BJH pore diameter V_{BJH} (Å)
MSN	999.19	1.03	31.49
MSN-SS-alkyne	692.24	0.64	27.91
DOX loaded MSN-SS-CD	399.69	0.40	/
DOX loaded MSN-SS-CD-Peptide-PASP	294.34	0.27	/



Scheme S1. Synthesis of MSN-SS-CD.



Scheme S2. Synthesis of PASP-alkyne.

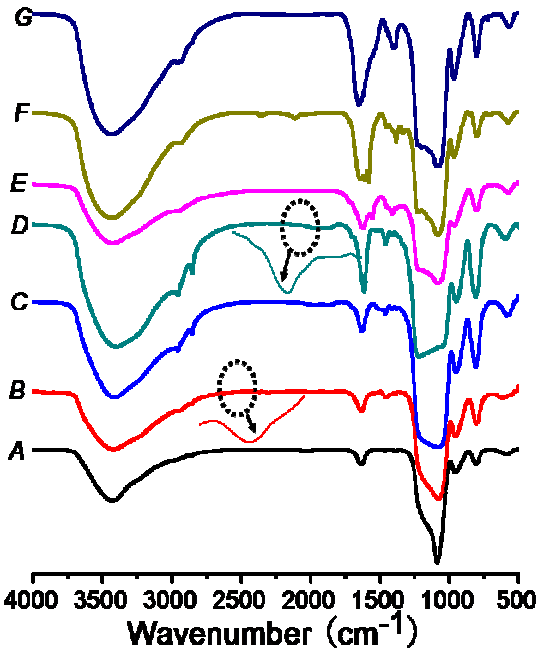


Figure S1. FT-IR spectra of MSN (A), MSN-SH (B), MSN-SS-NH_2 (C), MSN-SS-alkyne (D), DOX loaded MSN-SS-CD (E), DOX loaded MSN-SS-CD-Peptide (F) and DOX loaded MSN-SS-CD-Peptide-PASP (G).

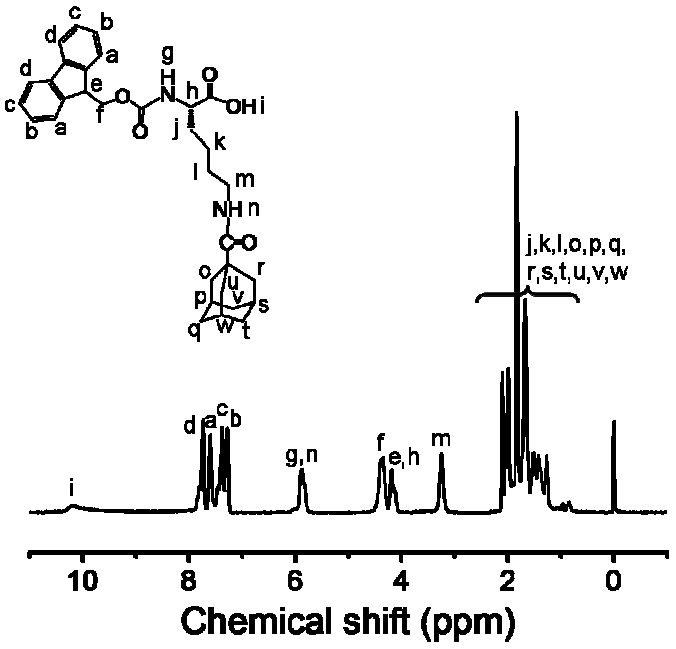


Figure S2. ^{1}H NMR spectrum of Fmoc-Lys(AD)-OH.

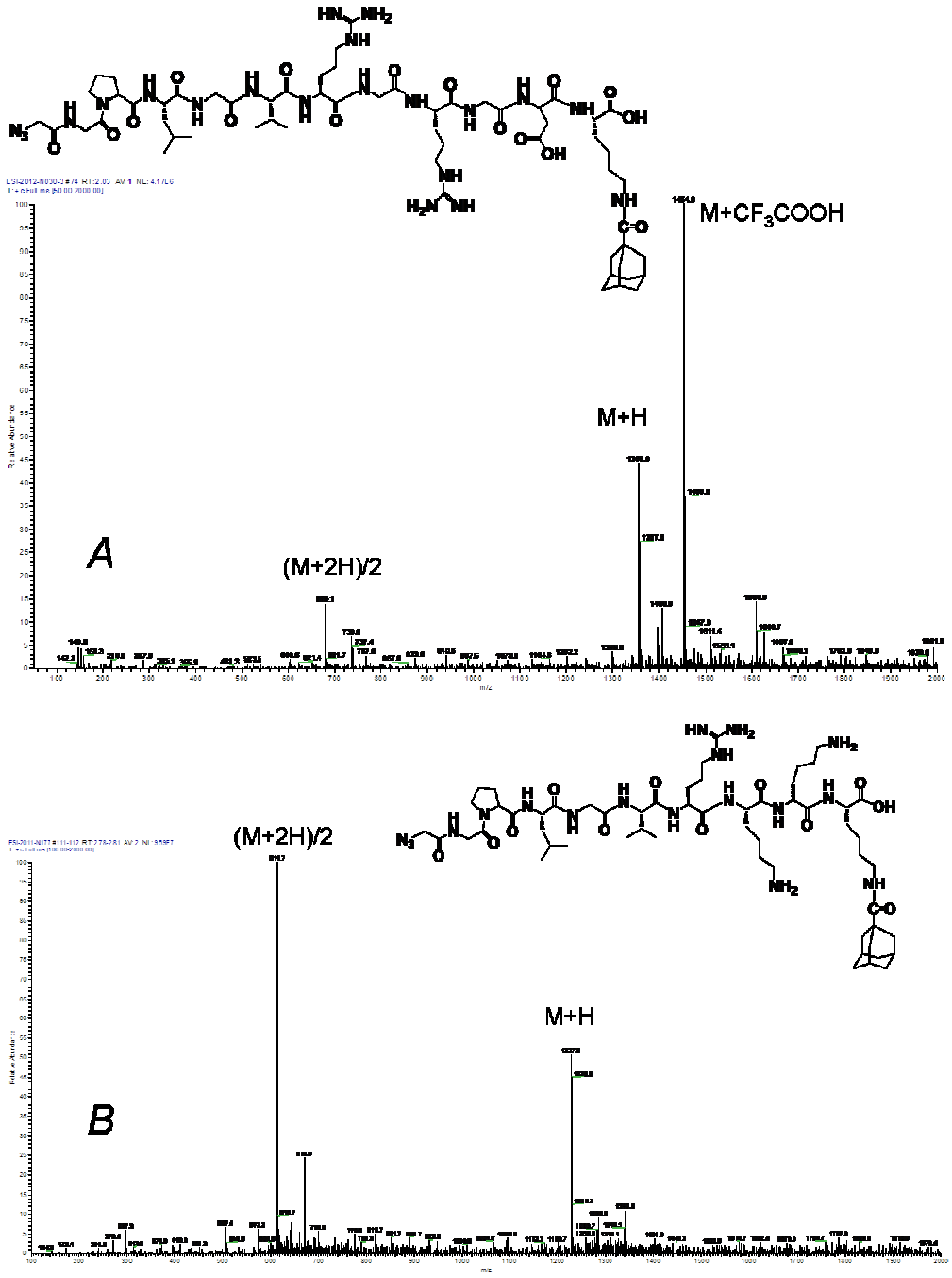


Figure S3. ESI-MS of N_3GPLGVRGRGDK-Ad (A) and N_3GPLGVRKKK-Ad (B).

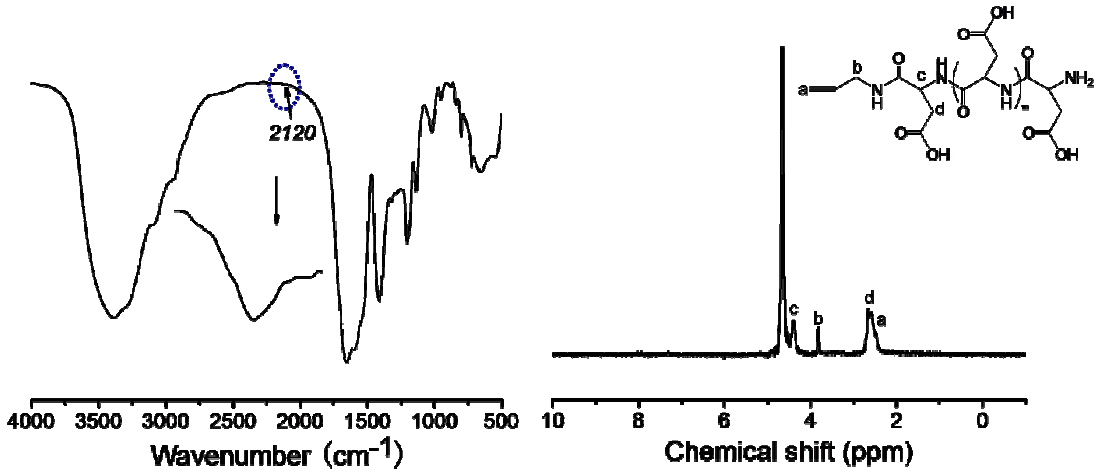


Figure S4. FT-IR spectrum and ^{1}H NMR spectrum of PASP-alkyne.

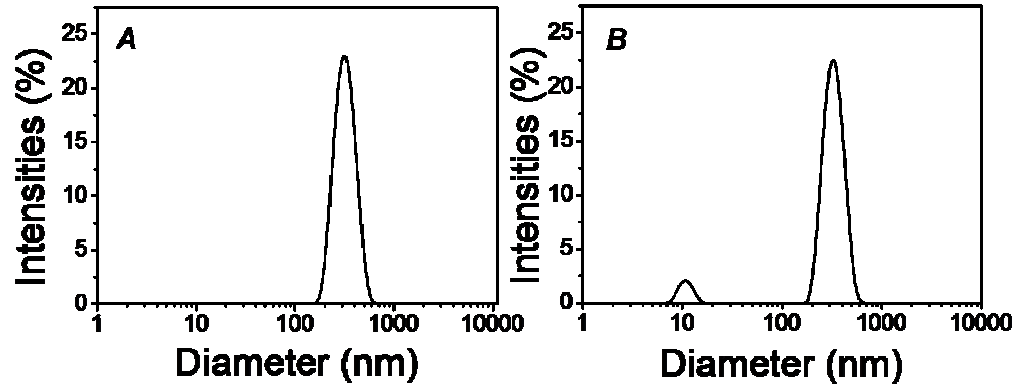


Figure S5. Size distribution of the DOX loaded MEMSN in DI water (A) and in PBS with 10% serum (B).

The small peak in Figure S5B was attributed to the protein in serum containing PBS, implying that the protein was not absorbed on MEMSN.

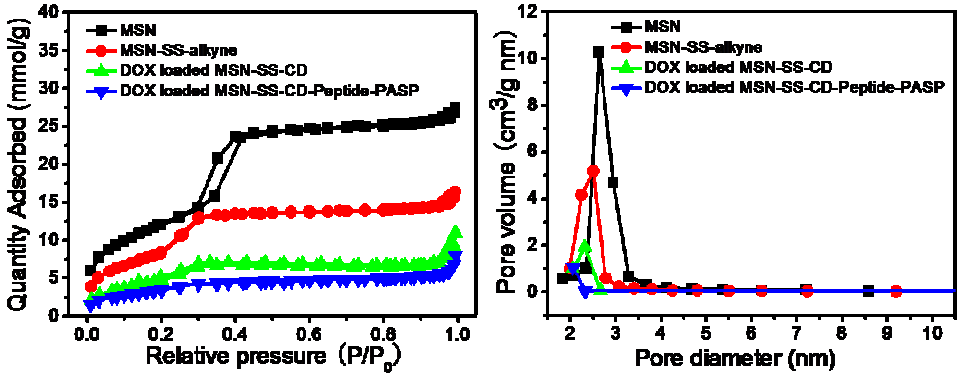


Figure S6. BET nitrogen adsorption/desorption isotherms (A) and BJH pore size distribution (B) of different nanoparticles.

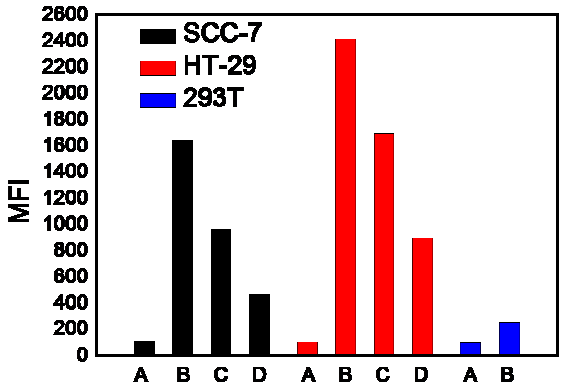


Figure S7. Flow cytometry analysis of SCC-7cells, HT-29 cells, and 293T cells; A) blank control; B) treated with DOX loaded MEMSN; C) treated with DOX loaded MSN-SS-CD-Peptide*-PASP; D) treated with DOX loaded MEMSN in the presence of MMP inhibitor.

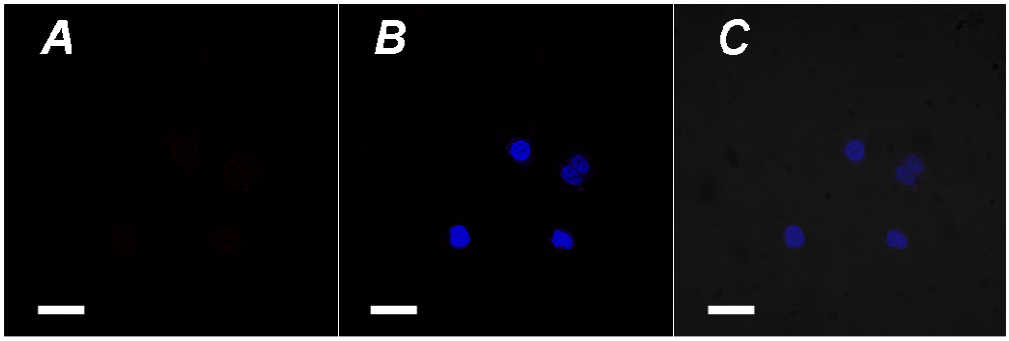


Figure S8. CLSM images of 293T cells treated by DOX loaded MEMSN. A) Red fluorescence images; B) confocal field images; C) overlap of confocal fluorescence and bright field images. (The scale bar is 30 μ m).

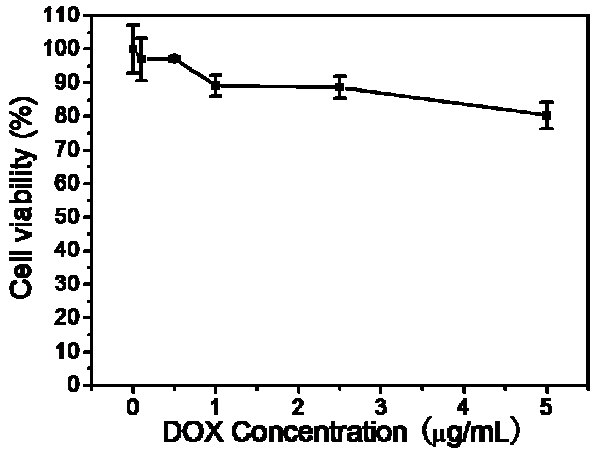


Figure S9. Viability of 293T cells after being incubated with DOX loaded MEMSN for 4 h.

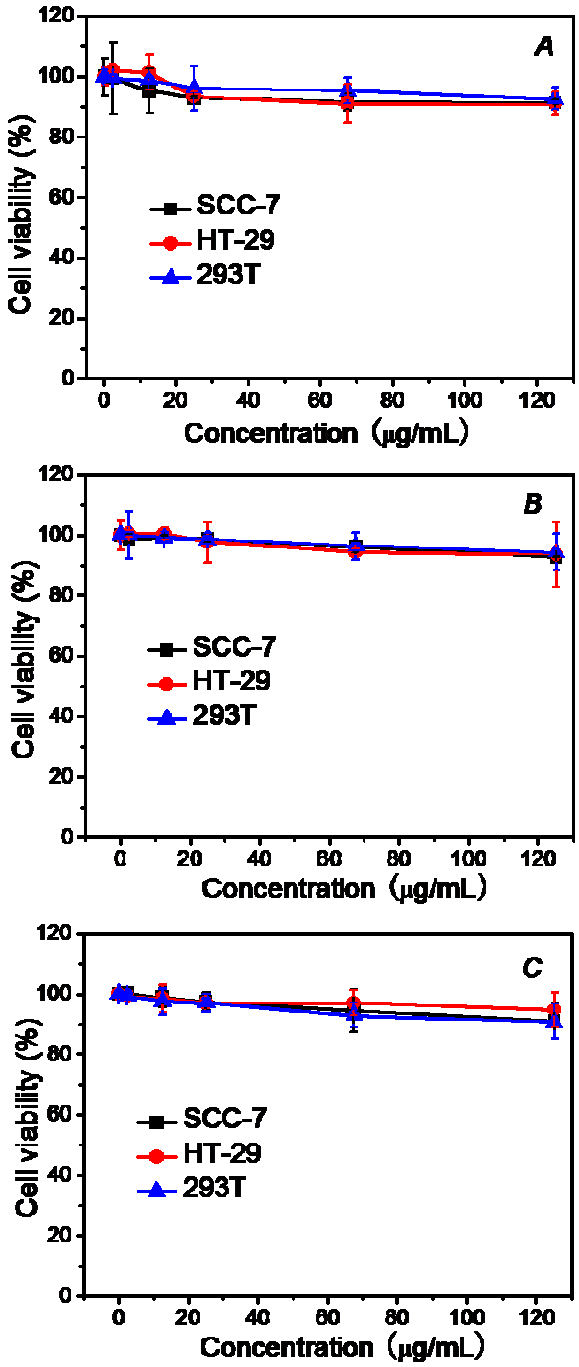


Figure S10. Viability of SCC-7 cells, HT-29 cells and 293T cells after being incubated with unloaded MSN-SS-CD-Peptide-PASP for 24 h (A), 48 h (B) and 72 h (C).

References

(S1) Ebright, Y. W.; Chen, Y.; Kim, Y.; Ebright, R. H. *Bioconjugate Chem.* **1996**, *7*, 380.

(S2) Qin, S. Y.; Xu, X. D.; Chen, C. S.; Chen, J. X.; Li, Z. Y.; Zhuo, R. X.; Zhang, X. Z. *Macromol. Rapid Commun.* **2011**, *32*, 758.