Electronic Supplementary Information

of

A redox-responsive mesoporous silica based nanoplatform for *in vitro* tumor-specific fluorescence imaging and enhanced photodynamic therapy

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Supplementary Expanded Methods

Reagents

Cetyltrimethylammonium bromide (CTAB), ammonium hydroxide (NH3·H2O), hydrochloric acid (HCl), 3-aminopropyltrimethoxysilane (APTMS), tetraethylorthosilicate (TEOS), ethanol, methanol, piperidine, acetic anhydride, dichloromethane (DCM), ether, dimethylsulfoxide (DMSO) and ninhydrin were purchased from Shanghai Reagent Chemical Co. and used as received. N,N-dimethylformamide (DMF) was purchased from Shanghai Reagent Chemical Co. and distilled prior to use. Rink amide resin (100–200 mesh, 0.77 mmol/g), N-fluorenyl-9-methoxycarbonyl (FMOC) protected L-amino acids (Fmoc-Gly-OH, Fmoc-Lys(dde)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Lys(N3)-OH), diisopropylethylamine (DIEA), triisopropylsilane (TIS), N-hydroxybenzotriazole (HOBt), o-benzotriazol-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) and trifluoroacetic acid (TFA) were obtained from GL Biochem Ltd and used as received. Black Hole Quencher N-Hydroxysuccinimide ester (BHQ-NHS), 3,3'-dithiodipropionic acid di(N-succinimidyl ester) (DSP) and PpIX were purchased from Aladdin Reagent Co. Ltd. Fmoc-$(PEG)_8$-COOH was received from Zhoubei Technology Co. Ltd. JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl benzimidazoly carbocyanine iodide) fluorescent dye was purchased from Qcbio Science & Technologies Co. Ltd. (China).

Cervical cancer cells (HeLa) were acquired from Cell Bank of Chinese Academy of Sciences (Shanghai, China). Dulbecco's phosphate buffered saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) ,

glutathione (GSH) and trypsin were purchased from Invitrogen. All other reagents and solvents were of analytical grade and used as received.

Characterization

The molecular weights of synthesized peptide were determined by electrospray ionization-mass spectrometry (ESI-MS, Finnigan LCQadvantage). The morphologies of MSNs and BHQ-MSN-SS-PpIX&RGD were observed by transmission electron microscopy (TEM, JEM-2100 microscope). The particle zeta potentials of MSNs in DI water were measured by using Malvern Zetasizer Nano-ZS ZEN3600. Fourier transform infrared spectroscopy (FTIR) was performed on a Perkin-Elmer spectrophotometer (USA) using potassium bromide (KBr) pellets. Ultraviolet–visible spectroscopy (UV-Vis) was measured on a PerkinElmer spectrometer (Lambda 35). Fluorescence analysis was carried out on a RF-5301PC spectrofluorophotometer (Shimadzu).

Synthesis of peptide analogs

The peptide $K(N_3)GK(PpIX)-(PEG)_8-RGD$ was synthesized in rink amide resin (100-200 mesh, loading: 0.77 mmol/g) through standard Fmoc-based solid phase peptide synthesis (SPPS). Briefly, Fmoc protected L-amino acid (4 equiv), HBTU (4 equiv), HOBt (4 equiv) and DIEA (6 equiv) were added to the resin in a DMF solution and reacted for 2 h. After repeated deprotection and acylation reaction, the peptide sequence was cleaved from resin with a mixture of TFA, TIS and distilled water in the volume ratio of 95:2.5:2.5 for 2 h. The final product was concentrated, precipitated in cold ether and dried under vacuum.

Synthesis of amino BHQ-doped mesoporous silica nanoparticles (BHQ-MSN-NH_2)

Briefly, 48 μL of APTMS was labeled with 3 mg BHQ NHS ester in 300 μL of N,N-dimethylformamide (DMF) solution containing 2% diisopropylethylamine (DIEA). The reaction mixture was stirred at room temperature for 6 h to obtain the APTMS-BHQ (Scheme S1a). Separately, 98.4 mg cetyltrimethylammonium bromide (CTAB) was dissolved in 70 mL of deionized water and 550 μL of $NH_3 \cdot H_2O$ (28%-30%) was added with magnetic stirring for 10 min at room temperature. Half of tetraethyl orthosilicate (TEOS) (0.72 mmol) was then added with vigorous stirring for 30 min. 50 μL of APTMS-BHQ in ethanol solution ($V_{APTMS}/V_{ethanol}$=1:3) was added, and the additional half of TEOS was added with vigorous stirring for 2 h. Another 100 μL of APTMS was added with vigorous stirring for another 2 h to obtain the BHQ-MSN-NH_2 (Scheme S1b). The resulting MSNs were collected by centrifugation and then washed three times with deionized water and ethanol. Unconjugated BHQs were completely removed through centrifugation steps. The MSNs were obtained by removing CTAB in acidic ethanol (1 mL of concentrated HCl in 50 mL of ethanol) for 24 h. The particles were washed three times with deionized water and then stored at 4 ^oC.

Surface modification of BHQ-doped mesoporous silica nanoparticles

200 mg BHQ-MSN-NH_2 nanoparticles obtained above was first modified with DSP (110 mg) in 25 mL DMF, and the mixture was stirred overnight at room temperature. After then, the BHQ-MSN-SS- nanoparticles were dissolved in 20 mL DMF solution containing 2-propynylamine (52 μL), and the mixture was stirred at room temperature overnight to obtain BHQ-MSN-SS-alkyne. Followed by adding K(N_3)GK(PpIX)-$(PEG)_8$-RGD peptide (50 mg) in DMF (15 mL) containing BHQ-MSN-SS-alkyne (50

mg), the mixture was stirred for 72 h at room temperature protected by nitrogen to obtain BHQ-MSN-SS-PpIX&RGD nanoparticles. After each modification step, the desired product was collected by centrifugation (10000 r/min, 10 min), washing with ethanol several times and drying under vacuum.

In vitro release studies

In vitro redox-responsive drug release experiment was carried out in 10 mM pH 7.4 PBS with 10 mM GSH. For example, 1 mg nanoparticles were suspended in PBS at a concentration of 1 mg/mL. The above solution was transferred to a dialysis bag (MWCO 14000 Da) and then immersed in 10 mL PBS containing 15 mg GSH, shaking at 120 rpm (37 °C). At predetermined time intervals, 3 mL released solution was replaced with an equal volume of fresh media, and the performance of PpIX release was monitored at 410 nm by using a RF-5301PC spectrofluorophotometer (Shimadzu). The release experiments were conducted in triplicate and the mean value of three independent experiments was obtained.

Intracellular uptake

The in vitro tumor-targeting specificity of BHQ-MSN-SS-PpIX&RGD was evaluated by cellular uptake assay. The $\alpha_v\beta_3$-positive HeLa cells were chosen as model cell lines. Cells were seeded in a glass bottom dish with 1 mL DMEM containing 1% antibiotics and 10% FBS, and incubated under a humidified 5% CO_2 atmosphere for 24 h. To investigate the receptor-mediated endocytosis and specific cellular uptake, HeLa cells were pre-treated with free RGD (5 μM) at 37 °C for 30 min. Then, 1mL DMEM containing BHQ-MSN-SS-PpIX&RGD were added into the plate, and the cells were further incubated for another 4 h.

Intracellular uptake also provides an estimate of whether higher GSH levels in the cytoplasm would enhance the drug release and the fluorescence recovery. HeLa cells were pre-treated with extra GSH (5 μM) at 37 °C for 30 min, and then washed by PBS for twice. DMEM (1mL) containing BHQ-MSN-SS-PpIX&RGD were added to the plate, and cells were further incubated for 4 h. After washing with PBS for several times, the cell nuclei was stained with blue molecular probe (Hoechst 33342) for 15 min. Subsequently, the cells were washed and transferred into fresh DMEM containing 10% FBS for observation under a laser scanning confocal microscopy (CLSM, Nikon C1-si TE2000, BD Laser).

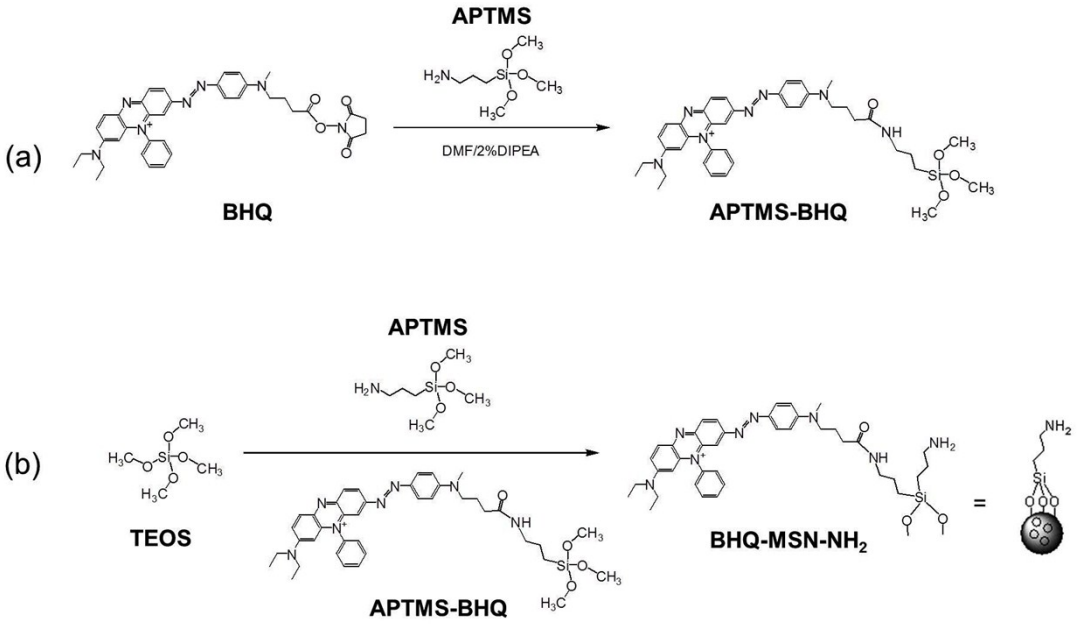
In vitro cytotoxicity

The tumor-selective acute cytotoxicity of BHQ-MSN-SS-PpIX&RGD was evaluated by MTT assay using normal cell lines (COS7 cells) and tumor cell lines (HeLa cells, SCC-7 cells). Briefly, cells were seeded at a density of 6000 cells/well in 96-well plates, and incubated with 100 μL DMEM containing 1% antibiotics and 10% FBS for 24 h at 37 ^{o}C. BHQ-MSN-SS-PpIX&RGD samples with various concentrations were added to each well and incubated for 4 h. Then, the medium was replaced with 200 μL fresh medium and light irradiation was performed for preset time (band pass: 400–700 nm, 3.3 mW cm $^{-2}$). After co-incubation for 48 h, 20 μL MTT solution (5 mg/mL) was added to each well and incubated for another 4 h. Finally, the medium was replaced with 150 μL DMSO, and the optical density (OD) values were measured by a microplate reader model 550 (BIO-RAD, USA) at 570 nm. The mean value of eight replicates was obtained as the final data for each sample, and the cell viability was calculated as follows: Cell Viability(%) = (OD_{sample} /$OD_{control}$) × 100, where $OD_{control}$ is relative to the media in the absence of samples and OD_{sample} is

relative to the media in the presence of samples.

Table S1 Zeta potentials of different nanoparticles in DI water.

Sample	Zeta potential (mV)
BHQ-MSN-NH_2	+24.0
BHQ-MSN-SS- succinimidyl	-13.4
BHQ-MSN-SS-alkyne	-2.30
BHQ-MSN-SS-PpIX&RGD	-5.18



Scheme S1 Synthesis procedure of (a) APTMS-BHQ and (b) BHQ-MSN-NH_2.

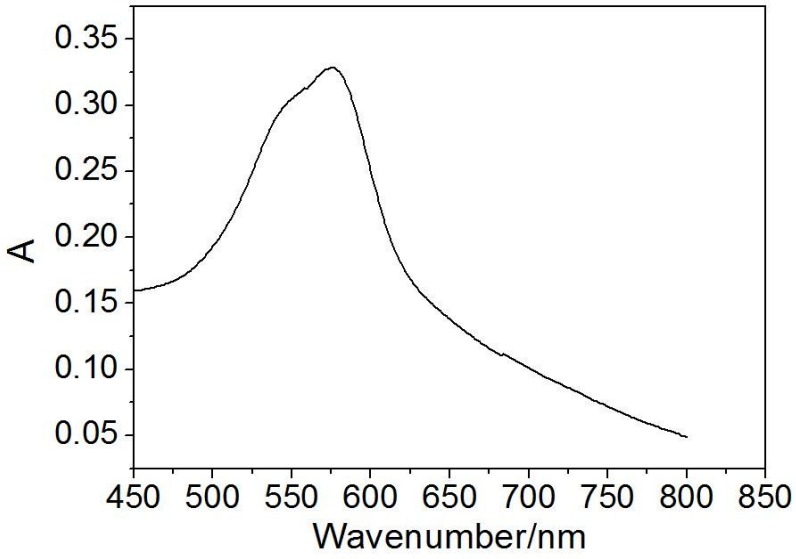


Fig. S1 UV-Vis spectrum of BHQ-MSN-NH_2.

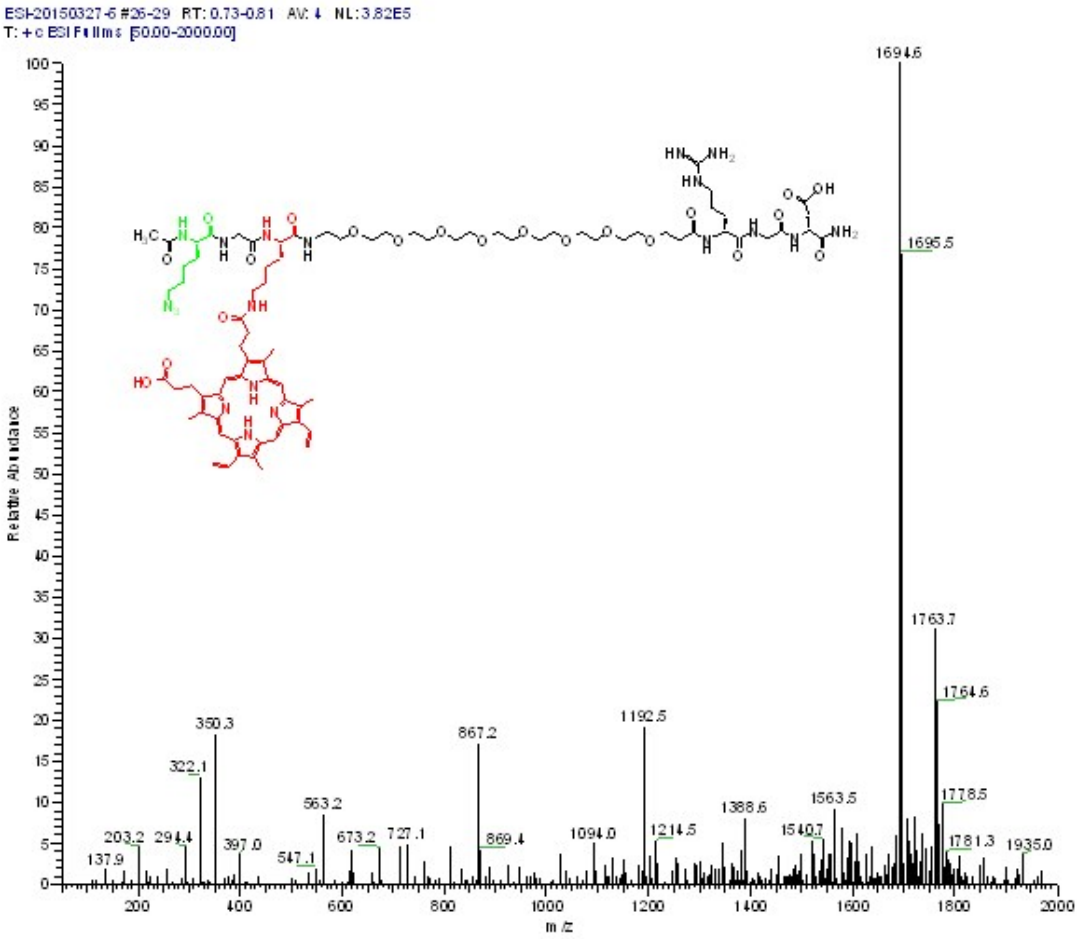


Fig. S2 ESI of multifunctional peptide.

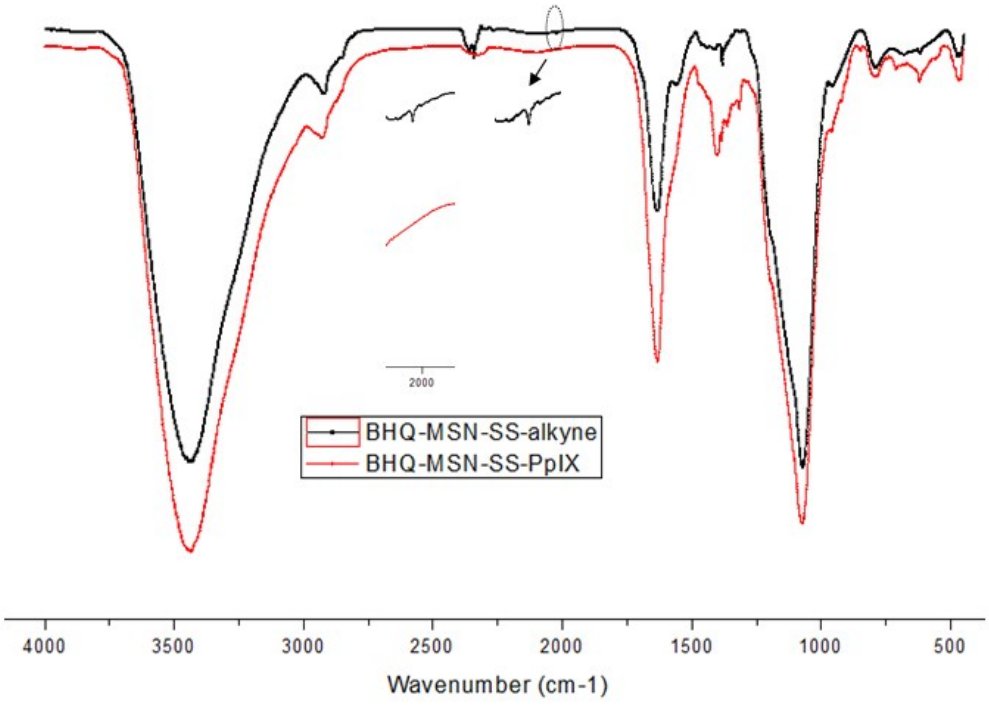


Fig. S3 FTIR spectra of BHQ-MSN-SS-alkyne (black) and BHQ-MSN-SS-PpIX&RGD (red).