

Electronic Supplementary Information

*of*

**Cucurbit[8]uril-mediated supramolecular photoswitching for self-preservation of mesoporous silica nanoparticle delivery system**

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Ning Ma, Wen-Jing Wang, Si Chen, Jing-Yi Zhu, Xiao-Qiang Wang, Shi-Bo Wang, Xiao-Shuang Wang, Si-Xue Cheng and Xian-Zheng Zhang\*

10 Key Laboratory of Biomedical Polymers of Ministry of Education & Department of Chemistry, Wuhan University, Wuhan 430072, P. R. China

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\*Corresponding author. xz-zhang@whu.edu.cn

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**Materials:** Cetyltrimethylammonium bromide (CTAB, 90%), tetraethylorthosilicate (TEOS), sodium ascorbate, methyl alcohol, 4, 4'-bipyridine, methyl iodide, 2-bromoethanoic acid, dichloromethane, glycoluril, paraformaldehyde, triethylamine (TEA), ethylene diamine tetraacetic acid (EDTA) and tris hydrochloride ultrapure (Tris) were purchased from Shanghai Reagent Chemical Co. (China) and used 5 as received. Trimethoxy[3-(amino) propyl]silane, 4-aminoazobene and succinic anhydride were purchased from Aladdin Reagent Co. Ltd. (Shanghai, China). N-Fluorenyl-9-methoxycarbonyl (Fmoc) protected L-amino acids (Fmoc-Lys-OH, Fmoc-Leu-OH, Fmoc-Glu-OH, Fmoc-Ile-OH, Fmoc-Thr-OH, Fmoc-Val-OH, Fmoc-Gly-OH, Fmoc-Arg(pbf)-OH, Fmoc-Pro-OH, Fmoc-Cys-OH, Fmoc-Tyr-OH, and Fmoc-Asn-OH), 2-chlorotriyl chloride resin (2-CTC resin) (100-200 mesh, loading: 0.537 10 mmol·g<sup>-1</sup>), N,N-diisopropylethylamine (DIEA), N,N'-diisopropylcarbodiimide (DIC), o-benzotriazole-N,N,N',N'-tetramethyluronium-hexafluoro-phosphate (HBTU), 1-hydroxybenzotriazole (HOBt) 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 15 triisopropylsilane (TIS) were purchased from Zhejiang Hisun Pharmaceutical Co. (China). Diisopropylethylamine (DIEA), pyridine, trifluoroacetic acid (TFA) and dimethylformamide (DMF) 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:** CTAB (0.5 g) was dissolved in deionized (DI) water (180 mL), and then NaOH 20 aqueous solution (70 mL, 2 M) was added and heated to 80 °C. TEOS (2.5 g) was added slowly to the solution under vigorous stirring. The reaction mixture was vigorously stirred at 80 °C for 2 h to obtain MSN.<sup>1</sup> The nanoparticles were then centrifuged (8000 r/min, 5 min), washed thoroughly with water and methanol for several times and dried under vacuum. The obtained nanoparticles were characterized by Transmission Electron Microscopy (TEM JEM-2100 HR) and scanning electron 25 microscopy (SEM, FEI-QUANTA 200).

**Synthesis of MSN-NH<sub>2</sub>:** MSN (0.7 g) was dispersed in methanol (55 mL). Then Trimethoxy[3-(amino)propyl]silane (1.5 mL) was added and the mixture was stirred for 24 h at room temperature to obtain MSN-NH<sub>2</sub> nanoparticles. The nanoparticles were then centrifuged (8000 r/min, 10 min), washed four times with methanol and dried under vacuum.

**5 Preparation of azobenzene-COOH:** 4-Aminoazobene (1.97 g, 10 mmol) and succinic anhydride (1.20 g, 12 mmol) were dissolved into 25 ml of distilled acetone. anhydrous pyridine (0.79 g, 10 mmol) was added into the solution and the mixture was stirred for 6 h at 60 °C.<sup>2</sup> The obtained suspension was filtered and dried at 40 °C for 48 h under vacuum to obtain azobenzene-COOH (azo-COOH) (yield: 97%).

**10 Synthesis of MSN-Azobenzene:** Azo-COOH (0.4 g, 1.35 mmol), DIC (0.22 ml, 1.35 mmol) and Hobt (0.192 g, 1.35 mmol) were dispersed into 10 ml of DMF. MSN-NH<sub>2</sub> (0.4 g) was added into the solution and the mixture was stirred for 24 h at room temperature. The nanoparticles were then centrifuged (8000 r/min, 10 min), washed several times with DMF and methanol, and then dried under vacuum. CTAB was removed by refluxing with a mixture of methanol (80 mL) and HCl (37.4 %, 4.5 mL) at 60 °C for 48 h. The nanoparticles were then centrifuged (8000 r/min, 10 min), washed four times with methanol and dried under vacuum.

**Synthesis of 1-methyl-4,4'-bipyridinium iodide:** Methyl iodide (4 mL) was added to a solution of 4,4'-dipyridyl (538 mg, 3.46 mmol). The mixture was heated to reflux for 1 h. Additional methyl iodide (5 mL) was added to reaction mixture.<sup>3</sup> Remaining methyl iodide was immediately rotary evaporated to prevent dimethylation and to obtain 1-methyl-4,4'-bipyridinium iodide (1.0159 g, yield: 99%) as an orange powder: <sup>1</sup>H NMR (300 MHz, MeOD) δ 9.06 (d, J=5.8 Hz, 2 H), 8.78-8.90 (m, 2 H), 8.52 (d, J=7.0 Hz, 2 H), 7.95-8.07 (m, 2 H), 4.38-4.60 (m, 3 H).

**Synthesis of CH<sub>3</sub>-NC<sub>6</sub>H<sub>4</sub>-C<sub>6</sub>H<sub>4</sub>NCH<sub>2</sub>CO<sub>2</sub>H:** 2-Bromoethanoic acid (0.48 g, 0.34 mmol) was mixed with 1-methyl-4,4'-bipyridinium iodide (0.61 g, 0.35mmol) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) and stirred for 2 days. The resulting precipitate was filtered, washed with CH<sub>2</sub>Cl<sub>2</sub> (2 × 5 mL), and dried in vacuo. The

precipitate was dispersed in  $\text{CH}_2\text{Cl}_2$  (20 mL) and stirred with triethylamine (2.5 mL) for 8 h.<sup>4</sup> The precipitate was filtered, washed with  $\text{CH}_2\text{Cl}_2$  ( $2 \times 5$  mL), and dried in vacuo to yield an orange powder, which was redissolved in water (5 mL) and evaporated at RT to give the title compound (yield: 48%). ESI-MS ( $\text{H}_2\text{O}$ ): 230  $[\text{M} + \text{H}]^+$ .  $^1\text{H}$  NMR (400.1 MHz,  $\text{D}_2\text{O}$ , ppm): 13.40 (s, H), 8.12 (d, 2H, J) 5.60 (s, 5 Hz), 8.01 (d, 2H, 5.60 Hz), 8.28 (d, 2H, 5.60 Hz), 7.79 (d, 2H, 5.60 Hz), 3.34 (s, 3H). IR ( $\text{cm}^{-1}$ ): 3451, 3022, 1635, 1547, 1457, 1416, 1333, 1180, 1077, 1052, 996, 907, 883, 848, 812, 755, 713, 678, 621.

**Synthesis of  $\text{CH}_3\text{-NC}_6\text{H}_4\text{-C}_6\text{H}_4\text{N-CH}_2\text{CO-GNYTCEVTEL TREGETI ELK}$  and  $\text{CH}_3\text{-NC}_6\text{H}_4\text{-C}_6\text{H}_4\text{N-CH}_2\text{CO-EVTEL TREGE}$ :** GNYTCEVTEL TREGETI ELK peptide (self peptide) and EVTEL TREGE (scrambled peptide) were manually synthesized in 0.537 mmol scale on the 2-10 chlorotriyl chloride resin, employing a standard Fmoc chemistry by solid phase peptide synthesis (SPPS) method. The coupling of the first residue used 3 equiv of Fmoc-protected amino acid (Fmoc-Lys-OH) relative to resin substitution degree with 6 equiv of DIEA in a DMF solution. Other amino acid couplings were carried out with 4 equiv of Fmoc-protecting amino acid, 6 equiv of DIEA and 4 equiv of HBTU and HOBt for 4 h.  $\text{CH}_3\text{-NC}_6\text{H}_4\text{-C}_6\text{H}_4\text{N-CH}_2\text{CO-GNYTCEVTEL TREGETI ELK}$  was synthesized by using 4 equiv of  $\text{CH}_3\text{-NC}_6\text{H}_4\text{-C}_6\text{H}_4\text{N-CH}_2\text{CO-OH}$ ,  $\text{N,N}'$ -dicyclohexylcarbodiimide (DCC) and HOBt mixed with deprotected peptide for 4 h.<sup>5</sup> During the synthesis, the Fmoc protecting groups were deprotected with 20 % (v/v) piperidine/ DMF twice. The cleavage of peptide was performed in a mixture of deionized water, TIS and TFA in the ratio of 2.5:2.5:95. After 2 h stirring at room temperature, the mixture was collected. The excess TFA was removed by rotary evaporation and the remaining viscous peptide solution was precipitated in the cold ether. The resulting orange product was collected and vacuum dried. The synthesis procedures  $\text{CH}_3\text{-NC}_6\text{H}_4\text{-C}_6\text{H}_4\text{N-CH}_2\text{CO-EGERTLETVE}$  were the same as  $\text{CH}_3\text{-NC}_6\text{H}_4\text{-C}_6\text{H}_4\text{N-CH}_2\text{CO-GNYTCEVTEL TREGETI ELK}$ . The molecular weight of  $\text{CH}_3\text{-NC}_6\text{H}_4\text{-C}_6\text{H}_4\text{N-CH}_2\text{CO-GNYTCEVTEL TREGETI ELK}$  ( $[\text{M}^+]$ ) measured by MALDI-TOP was 2610.29 (theoretical value: 2610.27). (1) 21 aa,  $\text{CH}_3\text{-NC}_6\text{H}_4\text{-C}_6\text{H}_4\text{N-CH}_2\text{CO-}$

GNYTCEVTELTREGETIIEELK (Self-peptide); (2) 10 aa, CH<sub>3</sub>-NC<sub>6</sub>H<sub>4</sub>-C<sub>6</sub>H<sub>4</sub>N-CH<sub>2</sub>CO-EGERTLETVE (Scrambled)

**Synthesis of CB[8]:** CB homologues were synthesized according to a reference procedure.<sup>6</sup> A mixture of glycoluril (28.4 g, 200 mmol), paraformaldehyde (12.0 g) and 150 ml hydrochloric acid was under 5 vigorous stirring for 30 min, heated at 75 °C for 24 h followed by 100 °C for 12 h, and then poured into acetone (2.0 L). The precipitate was washed with distilled water and hydrochloric acid (4 M) to obtain crystals of CB[8]. (120 mg, 2.9%). CB[8]: <sup>1</sup>H NMR (500 MHz, DCl/D<sub>2</sub>O (1:1)): δ 4.67 (d, 16H, J ) 15.5 Hz), 5.98 (s, 16H), 6.03 (d, 16H, J ) 15.5 Hz), 7.90 (s).

**Preparation of DOX·HCl loaded MSN-Azo-CB[8]-Dpy-Self peptide and MSN-Azo-CB[8]-Dpy-10 scrambled peptide/DOX·HCl:** MSN-Azo (200 mg) was stirred with DOX·HCl (32mg) in water (6 ml) for 24 h. The mixture was separated into two round-bottom flasks equally. Then two samples containing different peptides and CB[8] (a. self peptide: 40 mg, CB[8]: 20 mg; b. scrambled peptide: 18 mg, CB[8]: 20 mg) were dispersed homogeneously in DI water and added into above three flasks, respectively. The mixtures were stirred under nitrogen atmosphere for 20 h. The nanoparticles were 15 then centrifuged (5000 r/min, 10 min), washed thoroughly with water for several times and dried under vacuum.

**<sup>1</sup>H Nuclear Magnetic Resonance (<sup>1</sup>H NMR):** <sup>1</sup>H NMR spectra were recorded on a Mercury VX-300 spectrometer (Varian) at 300 MHz by using CDCl<sub>3</sub> or D<sub>2</sub>O as the solvent.

**Fourier Transform-Infrared Spectroscopy (FT-IR):** The samples in KBr pellets were analyzed by a 20 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 ZEN 3600 particle sizer (Malvern Instruments).

**Specific Surface Area of Porosity Analysis:** The surface area of porosity of the nanoparticles was measured by ASAP2020 Accelerated surface area and porosimetry system (Micromeritics).

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

**Ultraviolet spectrophotometry (UV):** The trans-cis photoisomerization of azobenzene was studied by UV/VIS spectrophotometry Lambda 35 (Perkin-Elmer) in aqueous solutions.

**5 In Vitro Drug Release:** Drug release from DOX·HCl loaded MSN-Azo-CB[8]-Dpy-Self peptide nanoparticles (10 mg) in phosphate buffer (PBS) were measured under different conditions (in dark and under UV, respectively) at 37 °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  $\lambda_{ex}=470$  nm.

**10 Flow Cytometry:** The quantitative evaluation of cellular uptake was performed by flow cytometry (BD FACSAria™ III). macrophage cells were seeded respectively in 6-well plates ( $5 \times 10^4$  cells/well) and cultured in DMEM (1 mL) containing 10% FBS for 24 h. After that, DOX·HCl-loaded nanoparticles (125 Pg/mL) dispersed in DMEM medium (1 mL) with 10% FBS and 1% antibiotics were added and the cells were further incubated at 37 °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 by centrifugation at 1500 rpm for 10 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 a negative control. The fluorescence scan was performed with  $1 \times 10^4$  cells.

**20 In Vitro Cytotoxicity:** The toxicity of the DOX·HCl loaded MSN-Azo-CB[8]-Dpy-Self peptide, DOX·HCl loaded MSN-Azo-CB[8]-Dpy-Scrambled peptide and Ultraviolet light DOX·HCl loaded MSN-Azo-CB[8]-Dpy-Self peptide in macrophages cells was evaluated by MTT assay. The cells were seeded respectively in a 96-well plate at a density of 6,000 cells/well and incubated in 100  $\mu$ L DMEM containing 10% FBS for 1 day. DOX·HCl loaded MSN-Azo-CB[8]-Dpy-Self peptide was treated by UV radiation for 30 min, and then the nanoparticles with particular concentrations were added co-

cultured with macrophages cells for 4 h. After that, the cell culture medium was removed and the medium containing DOX·HCl loaded nanoparticles was added. After incubated for 2 day, the medium was replaced with 200  $\mu$ L of fresh medium, then 20  $\mu$ L MTT (5 mg/ml) solutions was added to each well and further incubated for 4 h. Finally, the medium was removed and 200  $\mu$ L DMSO was added.

5 The absorbance at 570 nm was measured using a microplate reader (Bio-Rad 550). The relative cell viability was calculated as:  $\text{Viability} = (\text{OD}_{\text{treated}} / \text{OD}_{\text{control}}) \times 100\%$ , where  $\text{OD}_{\text{treated}}$  was obtained from the cells treated by a particular agent, and  $\text{OD}_{\text{control}}$  was obtained from the cells without any treatments. The data were given as mean  $\pm$  standard deviation (SD) based on four independent measurements.

## References

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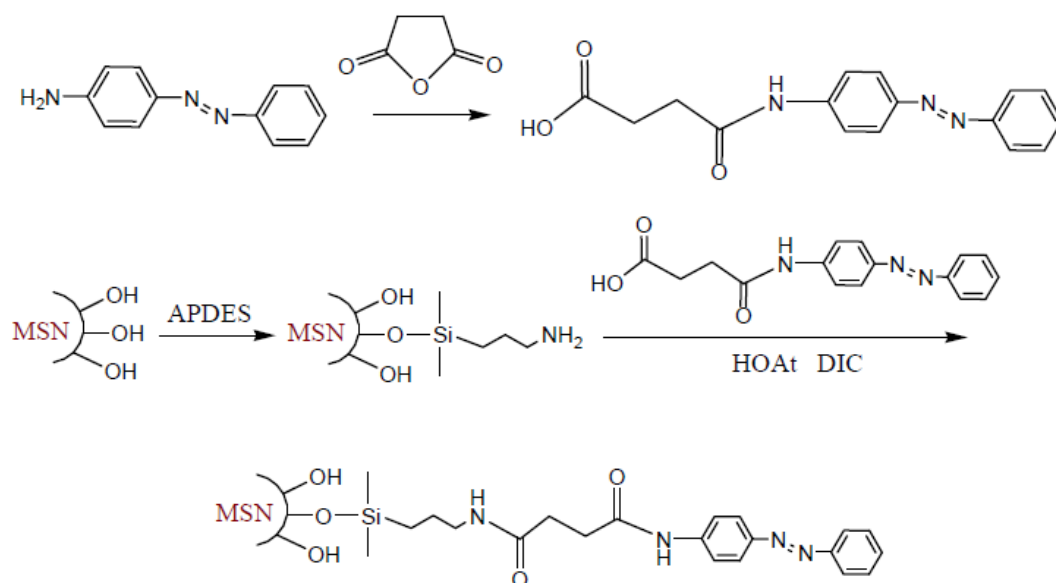
**Table S1.** Zeta potentials of different nanoparticles in DI water.

Sample	Zeta potential (mV)
MSN	-11.8
MSN-NH <sub>2</sub>	21.0
MSN-Azo	-11.7
DOX·HCL loaded MSN-Azo-CB[8]- Dpy-Self peptide	-20.3

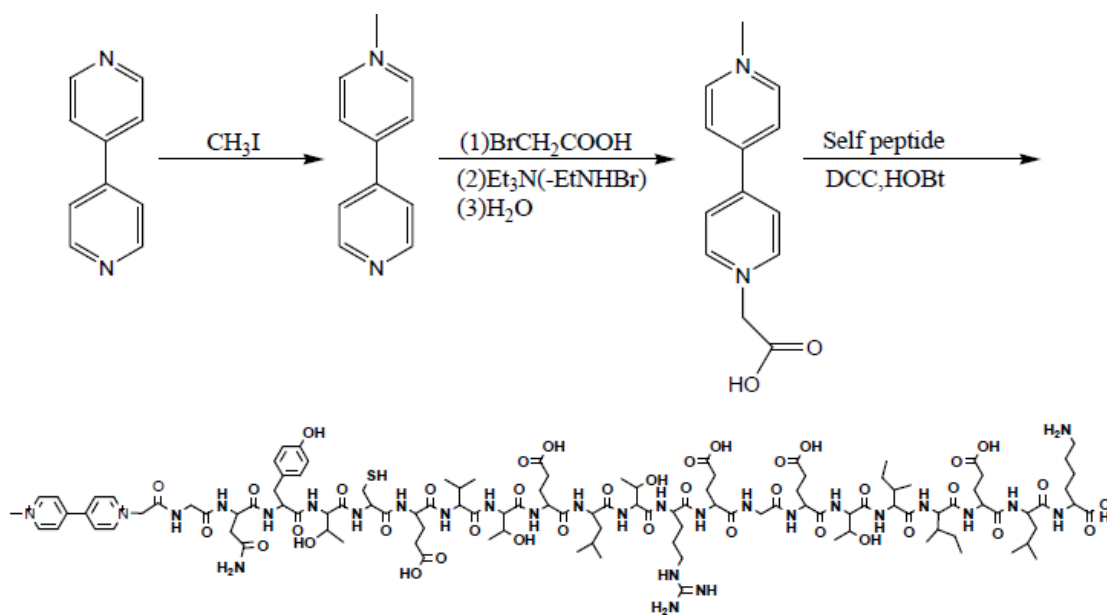
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**Table S2.** BET and BJH parameters of different nanoparticles

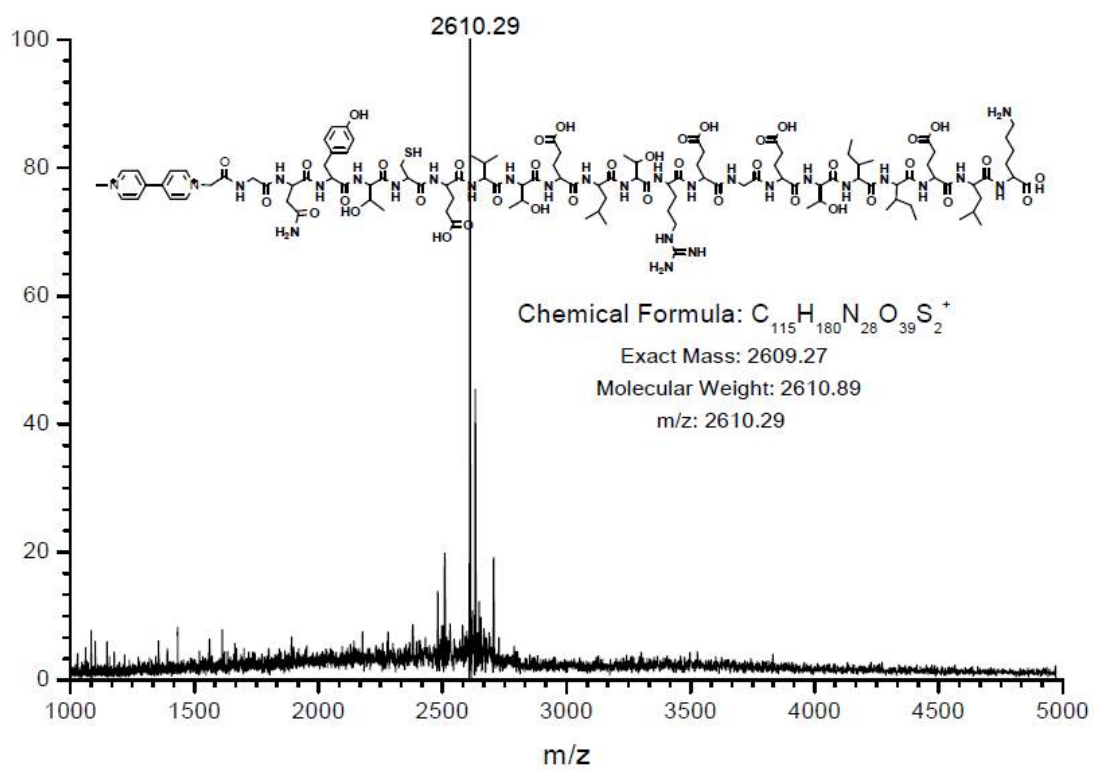
Sample	BET surface area S <sub>BET</sub> (m <sup>2</sup> /g)	BET pore volume V <sub>p</sub> (cm <sup>3</sup> /g)	BJH pore diameter V <sub>BJH</sub> (Å)
MSN	1090.96	1.13	35.14
MSN-NH <sub>2</sub>	745.85	0.68	30.34
MSN-Azo	605.93	0.32	29.35
DOX·HCL loaded MSN-Azo- CB[8]-Dpy-Self peptide	13.20	0.05	/



**Scheme S1.** Synthesis of the MSN-Azo.

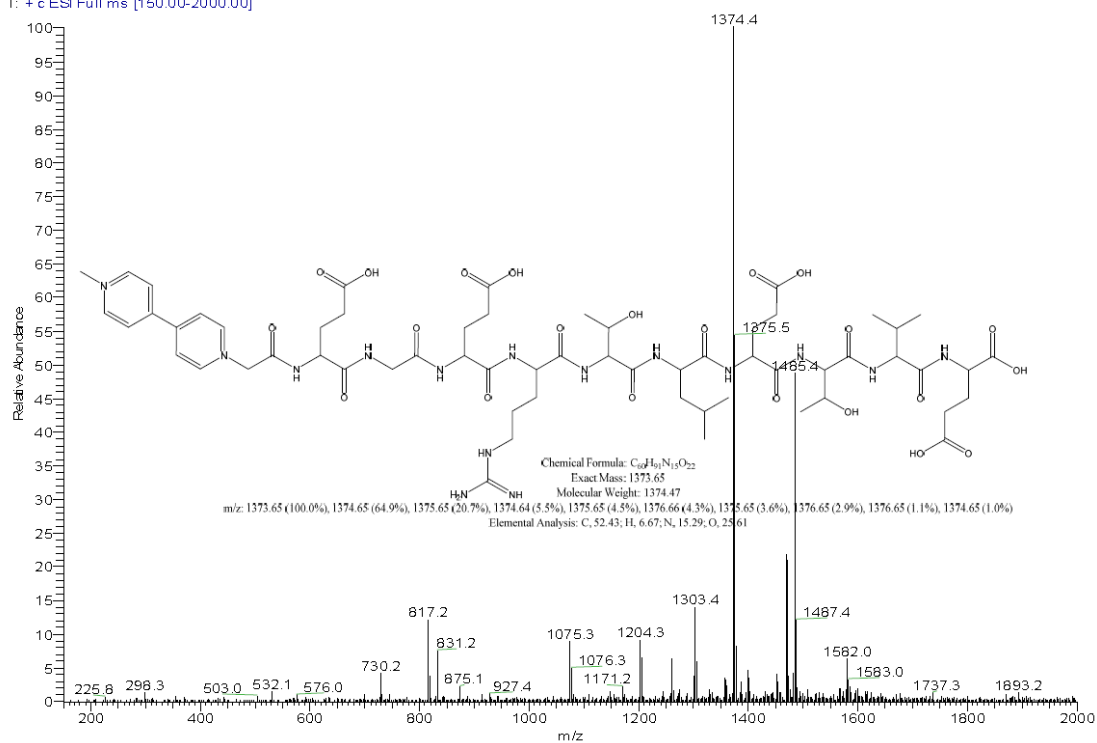


**Scheme S2.** Synthesis of Dpy-Self peptide.

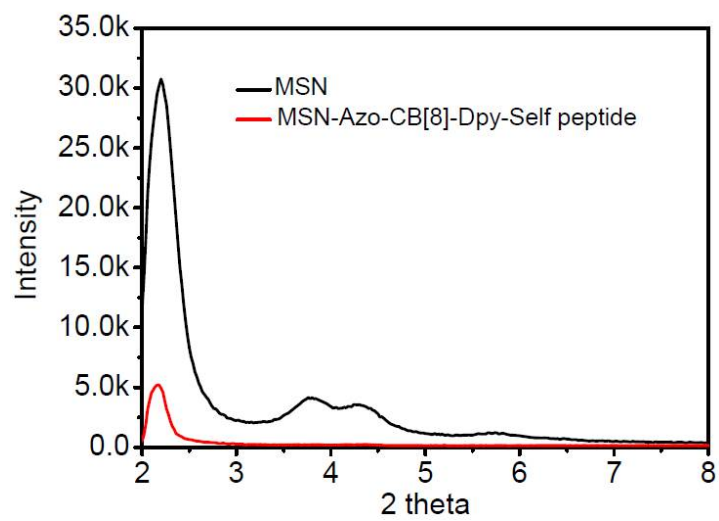


**Fig. S1.** MALDI-TOF of Dpy-Self peptide.

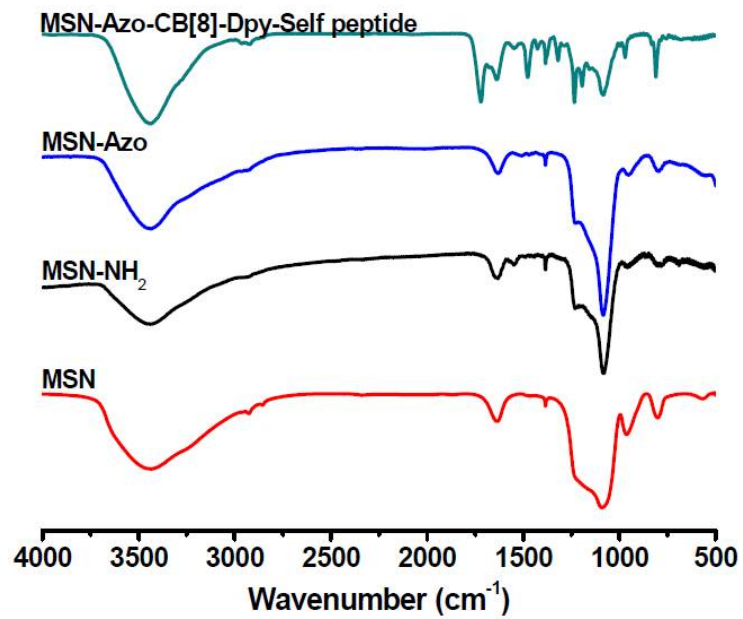
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T: + c ESI Full ms [150.00-2000.00]



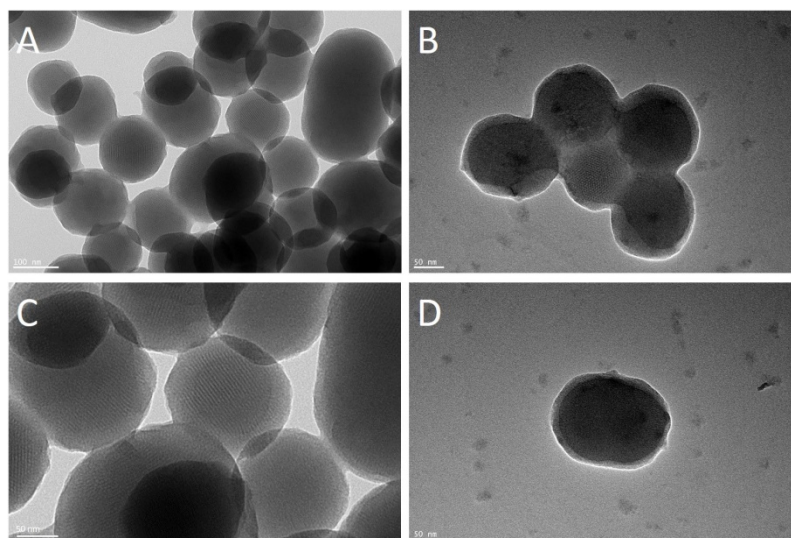
**Fig. S2.** ESI of Dpy-Scrambled peptide.



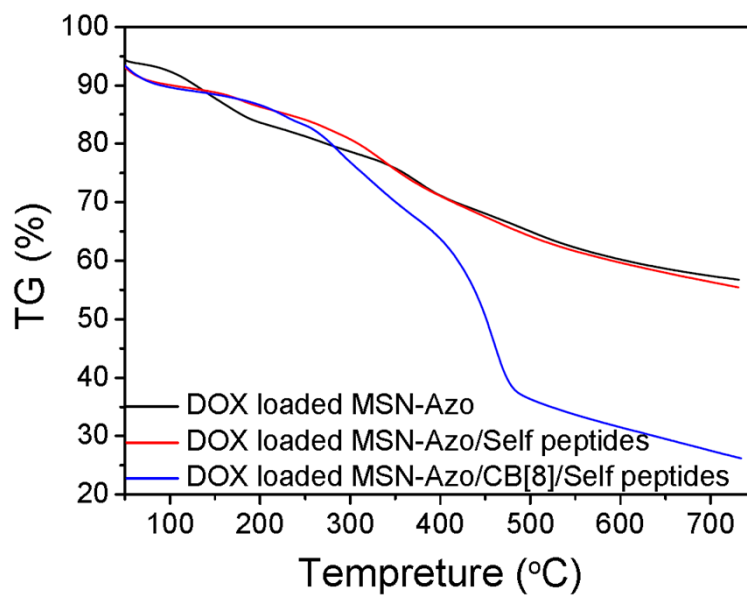
**Fig. S3.** PXRD of MSN and MSN-Azo-CB[8]-Dpy-Self peptide.



**Fig. S4.** FT-IR spectra of MSN; MSN-NH<sub>2</sub>; MSN-Azo and DOX·HCL loaded MSN-Azo-CB[8]-Dpy-

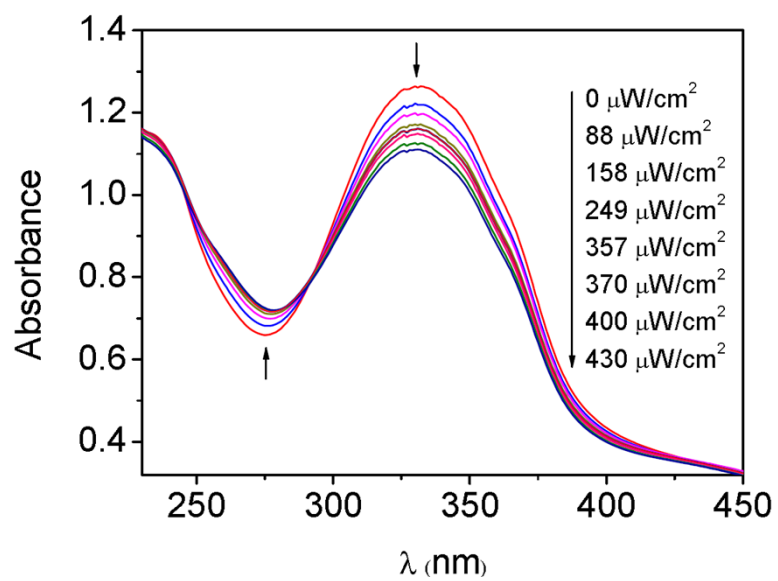


5 **Fig. S5.** (A) (C) TEM image of drug loaded MSN-Azo prepared through the same process without CB[8]. (B) (D) TEM image of drug loaded MSN-Azo-CB[8]-Dpy-Self peptide.

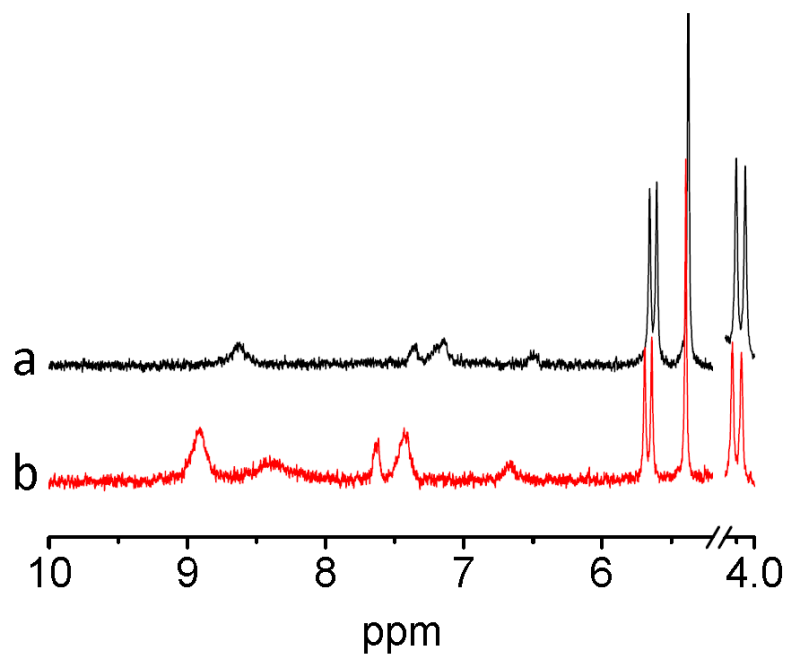


5 **Fig. S6.** TG of drug loaded MSN-Azo (black), TG of drug loaded MSN-Azo prepared through the same process without CB[8] (red) and TG of drug loaded MSN-Azo-CB[8]-Dpy-Self peptide (blue).





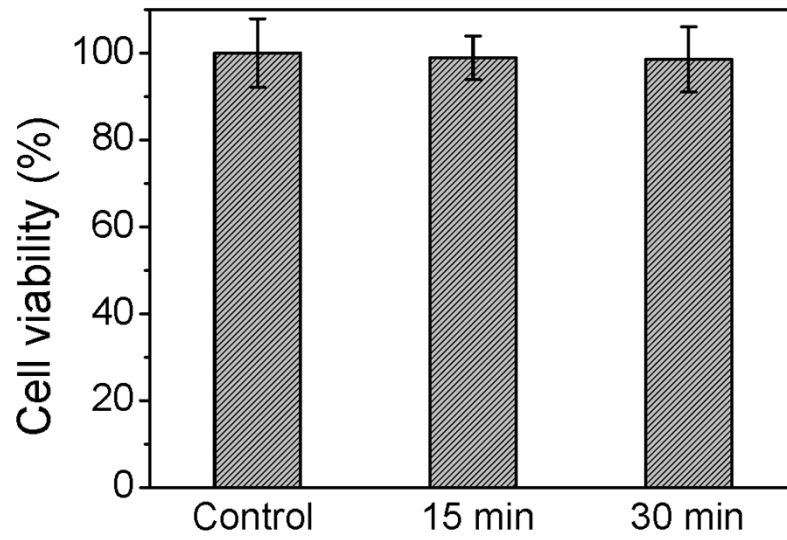
**Fig. S7.** UV/Vis spectra of MSN-Azo upon irradiation with UV light ( $\lambda=350$  nm; irradiated for 10 min) for different UV-intensity.



**Fig. S8.**  $^1\text{H}$  NMR spectra performed a mixture of 1:1:1 complex  $(\text{MV}^{2+*}\text{trans-1})\text{@CB[8]}$  at 1 mM (a), in  $\text{D}_2\text{O}$  at pH-7 at  $25^\circ\text{C}$  and irradiated under UV light for 30 min (b). The area around HOD peak

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between 4.2–5.2 ppm was removed for clarity



**Fig. S9.** In vitro cytotoxicity of THP1 macrophages exposed in UV light for 15 min and 30 min, determined by the MTT assay.