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for

Mechanized Silica Nanoparticles Based on Reversible Bistable [2]Pseudorotaxanes as Supramolecular Nanovalves for Multistage pH-Controlled Release

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1. Materials and Methods

Tetraethylorthosilicate (TEOS, ≥99.0%), (3-aminopropyl) trimethoxysilane (APTES, 97%), hexadecyltrimethylammonium bromide (CTAB, ≥98.0%), 4-aminopyridine (4-AP, ≥99.0%), di-*tert*-butyl dicarbonate (Boc_2O, ≥98.0%), 1,6-dibromohexane (96%), pyridine (≥99.0%), 2,2,2-trifluoroacetic acid (TFA, 99.0%), 2-propanol (99.5%), triethylamine (≥99.0%), 1*H*-benzotriazole (BTA, 99%) and cucurbit[6]uril (CB[6]) were purchased from Sigma-Aldrich. All the other reagents were of analytical grade and used as received. Phosphate buffered saline (PBS, pH 7.0) was used in the experiments. Fourier transform infrared spectra (FTIR) were recorded on a Bruker

Tensor 27 FTIR spectrometer. Transmission electron microscopy (TEM, JEM-2100, JEOL) was used to examine the morphology of the MSNs. Small-angle powder X-ray diffraction (SXRD) patterns were recorded on a Bruker D8 Advanced diffractometer using Cu Kα radiation (λ=1.5406 Å). The N_2 adsorption-desorption isotherms were obtained at 77 K on a Quanta chrome Nova 1000 Micrometric apparatus. Thermogravimetric analyses (TG) were performed using a Mettler TGA/SDTA 851e instrument with a heating rate of 10 ℃ min^{-1} under nitrogen flow. The solid state ^{13}C and ^{29}Si CP-MAS NMR measurements were collected on a Bruker DSX 400 NMR spectrometer, operating at Larmor frequency of 100.6 MHz and 79.5 MHz and equipped with a 7 mm probe. ^{1}H and ^{13}C NMR spectra were recorded using a Bruker DRX 500 spectrometer and tetramethylsilane (TMS) was used as a reference. UV/Vis spectroscopy was carried out with a Shimadzu UV-1800 spectrometer. The X-ray photoelectron spectra (XPS) of the powders were collected on a PHI QUANTERA Ⅱ X-ray photoelectron spectrometer, using a monochromatic Al Kα radiation (λ=8.4 Å) as the exciting source.

2. Preparation and Synthesis

2.1 Preparation of MSNs

A brief synthesis of MSNs was performed as follows: A mixture of 0.5 g CTAB, 1.75 mL NaOH (aq. 2.0 M) and 240 mL deionized H_2O was stirred vigorously at 80 ℃ for 1 h. After completely dissolution of CTAB, 2.5 mL TEOS was added dropwise to the surfactant solution. After a few minutes, a white solid were formed. The mixture was stirred at 80 ℃ with a stirring speed of 500 rpm for another 2 h. The as-synthesized materials were collected by filtration. The soft templates were removed by acid extraction method. The as-synthesized materials (0.5 g) were dispersed in the mixture

of 100 mL 2-propanol and 5 mL HCl (37%), and heated at 60 ℃ for 4 h. Finally, the resulting MSNs were filtered, washed with abundant H_2O and MeOH, and dried under vacuum overnight for further use.

2.2 Synthesis of Compound 1

(1) Synthesis of pyridine-4-yl-carbamic acid *tert*-butyl ester

***Scheme S1*.** Synthesis route of pyridine-4-yl-carbamic acid *tert*-butyl ester

To the mixture of 4-AP (0.26 g, 2.76 mmol) and Boc_2O (0.60 g, 2.75 mmol) was added distilled water (2.5 ml), and the reaction mixture was stirred magnetically at 35 ℃ for 1 h. After completion of the reaction, the residue separated by centrifugation was thoroughly washed with distilled water (3×5 ml) to afford the pyridine-4-yl-carbamic acid *tert*-butyl ester as a white solid (0.35 g, 65 %). ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm): 8.436 (d, *J*=6 Hz, 2H), 7.328 (d, *J*=6.5 Hz, 2H), 7.221 (s, 1H), 1.521 (s, 9H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 27.05, 80.10, 111.35, 145.40, 148.88, 151.32.

(2) Synthesis of 1-(6-bromohexyl)-pyridinium

***Scheme S2*.** Synthesis route of 1-(6-bromohexyl)-pyridinium

1,6-dibromohexane (9.15g, 37.5 mmol) was added into a 50 mL three-necked flask containing diethyl ether anhydrous (30 mL). Pyridinium (1.0 g, 12.6 mmol) was added dropwise to the above mixture under stirring at room temperature. After reacting for 40 h at 50 ℃, the solution was filtered to collect the resulting white precipitate. The white precipitate was recrystallized from ethanol/ether to give a white powder 1-(6-bromohexyl)-pyridinium (2.77 g, 68%). ^{1}H NMR (D_2O, 500 MHz) δ (ppm): 8.759 (d, *J*=5.5 Hz, 2H), 8.452 (t, *J*=7.5 Hz, 1H), 7.977 (t, *J*=7 Hz, 2H), 4.531

(t, *J*=7.5 Hz, 2H), 3.388 (t, *J*=6.5 Hz, 2H), 1.947 (q, *J*=7.5 Hz, 2H), 1.744 (q, *J*=7 Hz, 2H), 1.384 (q, *J*=7.5 Hz, 2H), 1.270 (q, *J*=7.5 Hz, 2H). ^{13}C NMR (D_2O, 500 MHz) δ (ppm): 23.28, 25.62, 29.29, 30.61, 33.92, 60.67, 127.16, 143.09, 144.47.

(3) Synthesis of Compound **1**

***Scheme S3*.** Synthesis route of Compound **1**

Pyridine-4-yl-carbamic acid *tert*-butyl ester (0.22 g, 1.133mmol) and 1-(6-bromohexyl)-pyridinium (0.32 g, 1.0 mmol) were dissolved in 15 mL acetonitrile. The mixture was heated under reflux for 48 h in an N_2 atmosphere. After cooling down to room temperature, the solvent was removed *via* rotary evaporation method and the residue was recrystallized from ethanol/petroleum ether to give an intermediate (0.33 g, 64%). Next step is to remove Boc group, the intermediate was dissolved in 1:1 TFA/CH_2Cl_2 (12 mL) in ice bath. The reaction was allowed to stand for 2 h at room temperature. The solution was concentrated, and the resulting residue was recrystallized from ethanol/ethyl acetate and dried under vacuum to yield of compound **1** (0.24 g, 71%). ^{1}H NMR (D_2O, 500 MHz) δ (ppm): 9.066 (d, *J*=6 Hz, 2H), 8.516 (t, *J*=8 Hz, 1H), 8.033 (t, *J*=7 Hz, 2H), 7.933 (q, *J*=7.5 Hz, 2H), 6.801 (d, *J*=7.5 Hz, 2H), 4.572 (t, *J*=7.5 Hz, 2H), 4.085 (t, *J*=7 Hz, 2H), 1.980 (t, *J*=7 Hz, 2H), 1.810 (t, *J*=7 Hz, 2H), 1.312 (t, *J*=3.5 Hz, 4H). ^{13}C NMR (D_2O, 500 MHz) δ (ppm): 23.62, 28.46, 29.07, 56.77, 60.56, 108.58, 126.99, 141.27, 142.94, 144.35, 157.44.

2.3 Synthetic procedure for preparing MSNPs 1

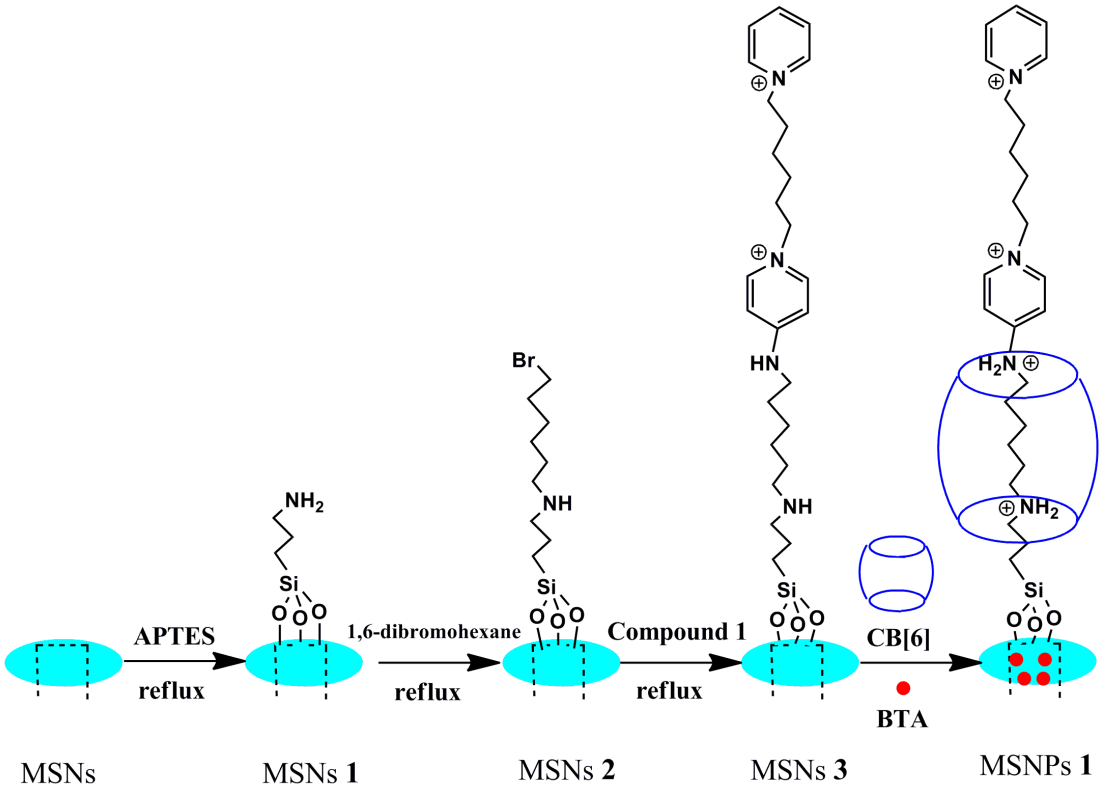

Figure S1. Synthetic procedure for preparing MSNPs **1**

2.4 Preparation of MSNs 1

MSNs (200 mg) were suspended in anhydrous toluene (15 mL) with the aid of ultrasound. APTES (100 μL, 0.43mmol) was added to the solution and the suspension was stirred under refluxing in the N_2 atmosphere overnight. After this, the MSNs **1** were separated by centrifugation, washed with anhydrous toluene (3×10 mL) and methanol (3×10 mL), and dried under vacuum at 70 ℃ overnight.

2.5 Preparation of MSNs 2

MSNs **1** (200 mg) were suspended in anhydrous toluene (15 mL), and 1,6-dibromohexane (100 μL, 0.62 mmol) was added to the solution, followed by heating at reflux for 24 h in the N_2 atmosphere. The nanoparticles were then centrifugated, washed with anhydrous toluene (3×10 mL) and methanol (3×10 mL) and dried under vacuum at 70 ℃ overnight to obtain MSNs **2**.

2.6 Preparation of MSNs 3

MSNs **2** (200 mg), Compound **1** (100 mg, 0.19 mmol) and triethylamine (100 μL, 0.72 mmol) were suspended in acetonitrile (15 mL) and the resultant mixture was stirred at 70 ℃ under an atmosphere of N_2 for 24 h. The MSNs **3** were isolated by centrifugation, washed with acetonitrile (3×10 mL) and methanol (3×10 mL) and dried under vacuum at 70 ℃ overnight.

2.7 Preparation of MSNPs 1

MSNs **3** (50 mg) were added to the BTA aqueous solution (15 mg mL^{-1}) followed by sonicating for 10 min and stirring at room temperature for 24 h. The BTA-loaded MSNs **3** were obtained by centrifugation and dried under vacuum overnight. After loading, the PBS solution (pH 7.0, 5 mL) containing CB[6] (50 mg), NaCl (5 mg) and BTA (10 mg) were prepared. BTA-loaded MSNs **3** were suspended in the above solution, and the mixture solution was stirred for 3 d at room temperature. The MSNPs **1** were collected by centrifugation, washed adequately with PBS solution (pH 7.0) and MeOH, and subsequently dried under vacuum overnight.

3. Controlled Release Experiments

In order to investigate the operation modes of MSNPs **1** in aqueous solution, UV/Vis spectroscopy was used to determine the concentration of BTA in solution released from MSNPs **1**.

Mode 1: Alkaline-triggered controlled release experiments

MSNPs **1** (1 mg) were placed in the dialysis membrane at the top of the quartz cuvette to avoid interfering light intensity. The PBS solution (4 mL, pH 7.0) was carefully added into the cuvette in order to ensure that MSNPs **1** were completely immersed into the solution. The MSNPs **1** were activated by adjusting the aqueous solution to the desired pH with NaOH solution (0.1 M). Release profiles were recorded by plotting the absorbance intensity of BTA in solution at 265 nm at one second interval as a function of time.

Mode 2: Multistage pH-controlled release experiments

MSNPs **1** (1 mg) placed in the dialysis membrane were immersed in the PBS solution (4 mL, pH 4.5). A few minutes later, the pH of solution was adjusted to 9.5 by addition a few drops of NaOH solution (0.1 M). After releasing BTA for 1 h, the pH of solution was changed to 4.5 by addition of HCl solution. Immediately afterwards, the suspension in quartz cuvette was taken out of the UV/Vis spectrophotometer and heated at 45 ℃ for 2 h. The UV/Vis absorbance was continuously monitored in the next 2 hours. Finally, the supramolecular nanovalves were opened again by reverting the pH value (pH=9.5) to release remaining BTA molecules.

Mode 3: Assessment of recyclable MSNPs **1**

MSNPs **1** (1 mg) placed in the dialysis membrane were immersed in the alkaline solution (4 mL, pH 10.5) for 12 h in order to release adsorbed BTA as many as possible. The empty MSNPs **1** were collected by filtration and soaked in the BTA aqueous solution (15 mg mL^{-1}, pH 10.5) for 24 h. After that, the closure of nanovalves was realized by adjusting the pH of solution to the 4.5. Finally, the BTA-reloading MSNPs **1** were recovered and performed for the second alkaline-triggered controlled release experiment.

4. Analytical data

4.1 Characterization of MSNs

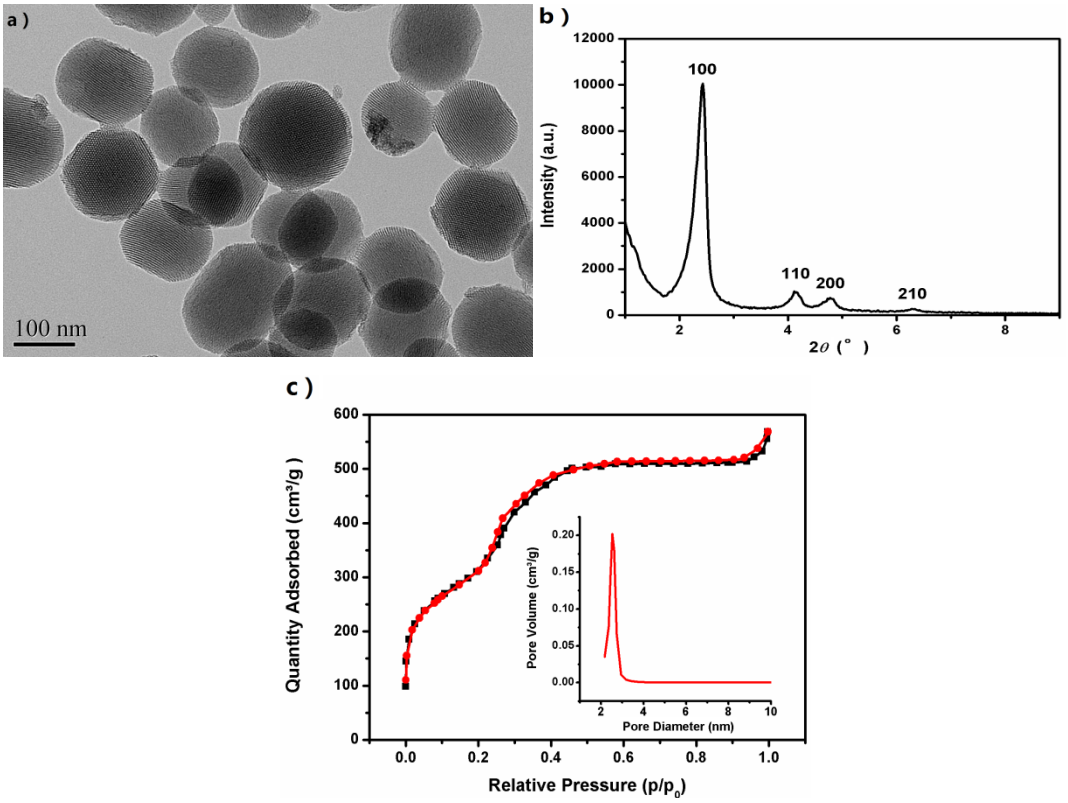

Figure S2. a) TEM image, b) Small-angle X-ray diffraction pattern, and c) N_2 adsorption-desorption isotherm and pore size distribution of MSNs

The SXRD pattern of MSNs in Figure S2b shows four reflections that can be assigned as (100), (110), (200), and (210), typical of ordered two-dimensional hexagonal mesostructure. This is also confirmed by TEM observations in Figure S2a. The N_2 adsorption-desorption isotherm of MSNs in Figure S2c exhibits a type Ⅳ isotherm with a hysteresis loop. The specific surface area is calculated by the Brunauer-Emmett-Teller (BET) method, and the pore size and pore volume are calculated by the Barrett-Joyner-Halenda (BJH) method according to the adsorption branch of the isotherm, which are all listed in Table S1.

Table S1. Physicochemical properties of the MSNs

Materials	Specific surface area	Pore size	Pore volume

	(m^2 g^{-1})	(nm)	(cm^3 g^{-1})
MSNs	1118	2.65	0.65

4.2 ^{29}Si CP-MAS Solid-State NMR spectra

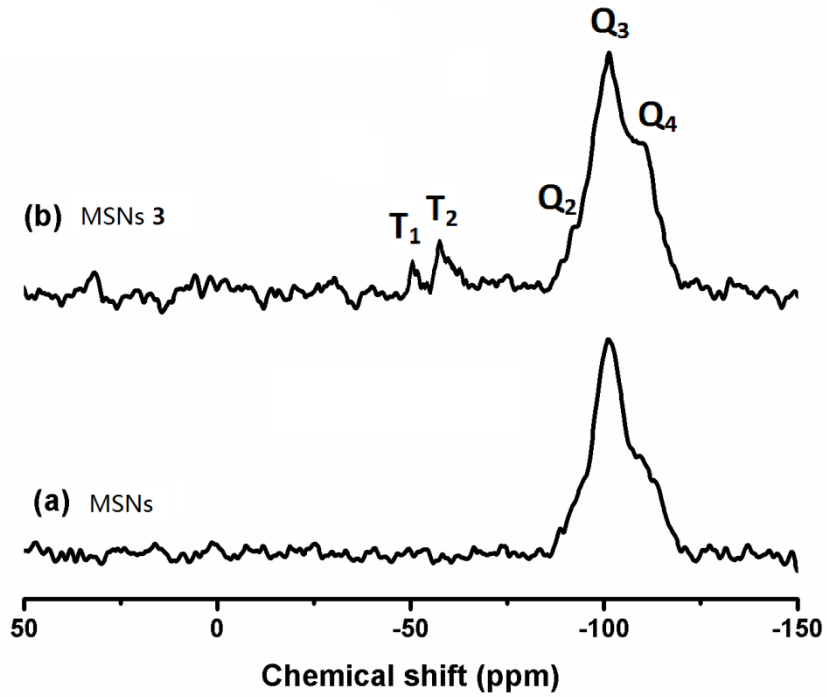

Figure S3. ^{29}Si CP-MAS Solid-State NMR spectra of pure MSNs (a) and MSNs **3** (b)

The ^{29}Si CP-MAS spectrum of MSNs shows three signals in the Q region at -93, -101, and -110 ppm, which corresponds to Q_2 ($Si(OSi)_2(OH)_2$), Q_3 ($Si(OSi)_3(OH)$), and Q_4 ($Si(OSi)_4$) silica species, respectively. The spectrum of MSNs **3** shows the additional T signals at -51 (T_1) and -56 ppm (T_2), confirming the formation of Si-C bonds.

4.3 XPS analyses

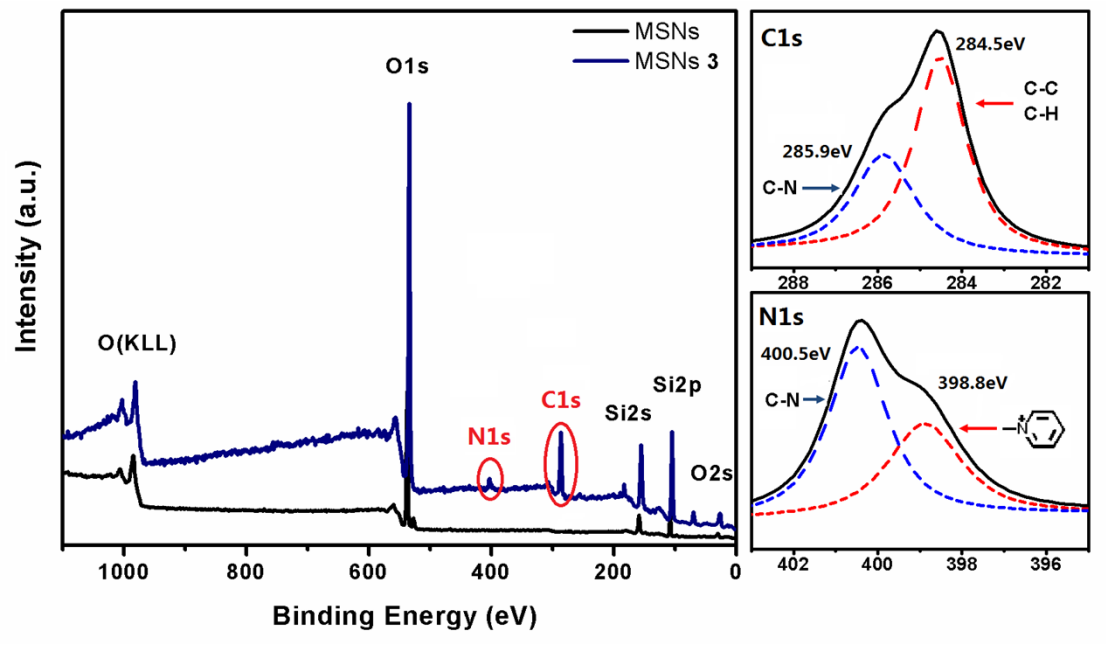

Figure S4. (a) wide-scan XPS spectra of MSNs and MSNs **3**, (b) XPS high-resolution C1s core line spectrum of MSNs **3**, and (c) XPS high-resolution N1s core line spectrum of MSNs **3**

The C1s peak of MSNs **3** can be deconvoluted into two binding energies at 284.5 eV (-C-H or –C-C-) and 285.9 eV (C-N). Likewise, the N1s peak can also be divided into the two components. The component at 398.8 eV is assigned to the nitrogen atoms of pyridine rings and the other component at 400.5 eV corresponds to the nitrogen atoms of aliphatic amine.

4.4 TG analyses

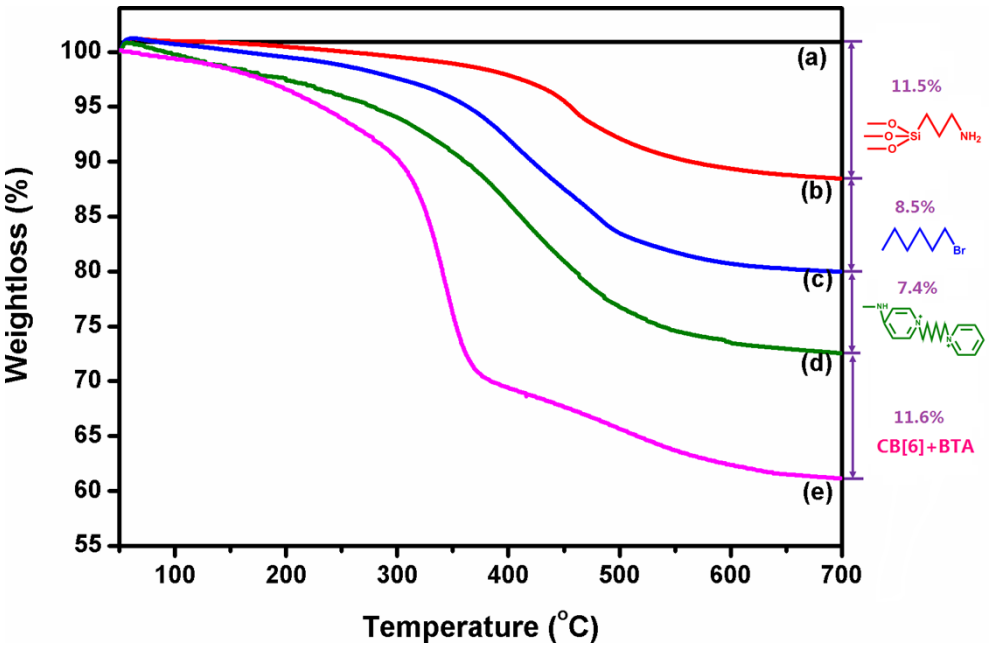

Figure S5. TGA analyses of a) MSNs, b) MSNs **1**, c) MSNs **2**, d) MSNs **3**, and e) MSNPs **1**

4.5 XRD and N_2 adsorption-desorption isotherms analyses

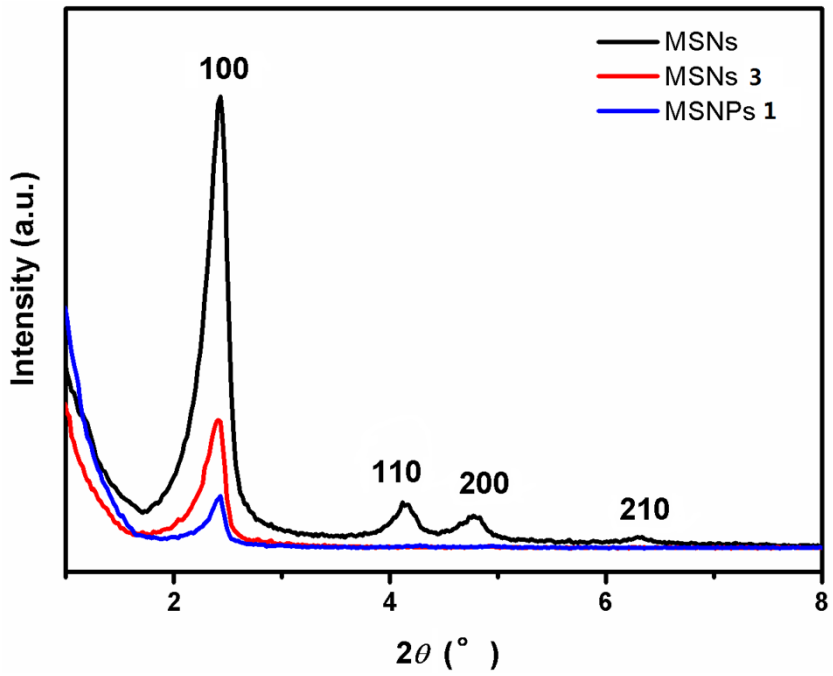

Figure S6. Small-angle X-ray diffraction pattern of MSNs (blank line), (b) MSNs **3** (red line), and (c) MSNPs **1** (blue line).

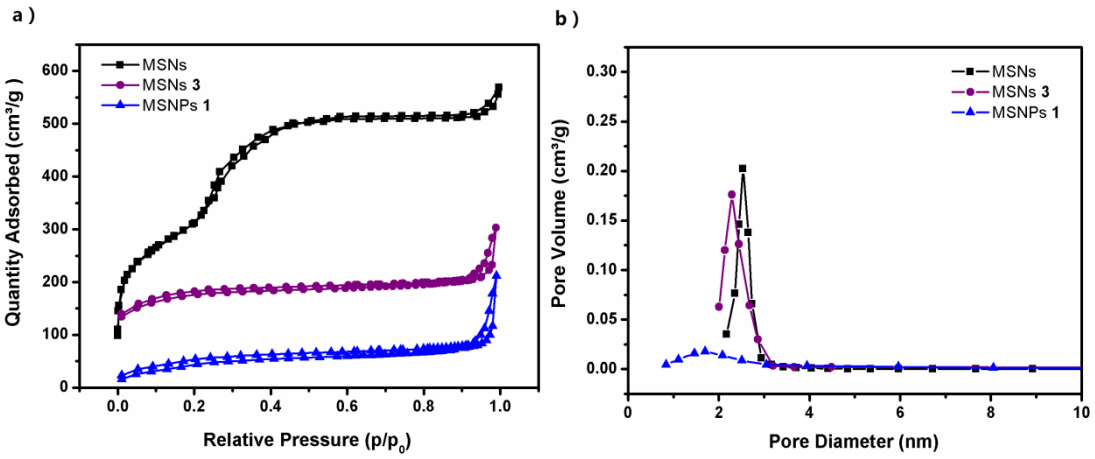

Figure S7. N_2 adsorption-desorption isotherms (a) and pore size distributions (b) of MSNs, MSNs **3** and MSNPs **1**

Table S2. Physicochemical properties of the MSNs, MSNs **3**, and MSNPs **1**

Materials	**Specific surface area** ($m^2\ g^{-1}$)	**Pore size** (nm)	**Pore volume** ($cm^3\ g^{-1}$)
MSNs	1118	2.65	0.65
MSNs 3	427	2.29	0.34
MSNPs 1	150	–	0.09

Through a comparative analysis, the intensity of the diffraction peaks declines obviously after three-step functionalization. However, MSNs **3** as well as MSNPs **1** still maintain characteristic reflection (100) peak. These phenomena mean that all the procedures including whole functionalization, loading with BTA and capping with CB[6] macrocycles do not fairly destroy the mesoporosity structure. Moreover, for MSNs **3**, the clear reduction of specific surface area, pore size and pore volume was observed as expected due to the fact that the mesoporous channels are partially occupied by the organic stalks.

4.6 Determination of the loading amount of BTA

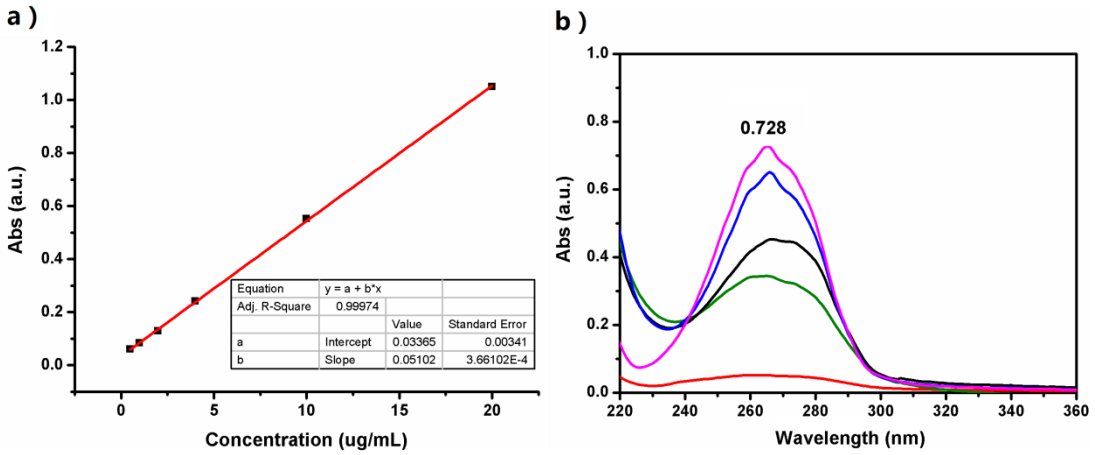

Figure S8. a) Calibration curve of UV/Vis absorption intensity of BTA and b) The UV/Vis absorption spectra of BTA released from MSNPs **1** completely (purple line), under pH 10.0 for 6 h (blue line), under pH 9.0 for 6 h (black line), under pH 8.0 for 6 h (green line) and under pH 7.0 for 6 h (red line)

In order to evaluate the all sorts of pH-controlled release performances of MSNPs **1**, the primary task is to determine the equilibrium adsorbed amount of BTA within MSNPs **1**. It is calculated by UV/Vis absorption measurement of the supernatant of the MSNPs **1** sample under pH=10.0 after 12 h sonication and the value is 25.9 mg BTA g^{-1} MSNPs **1**.

4.7 Control experiments

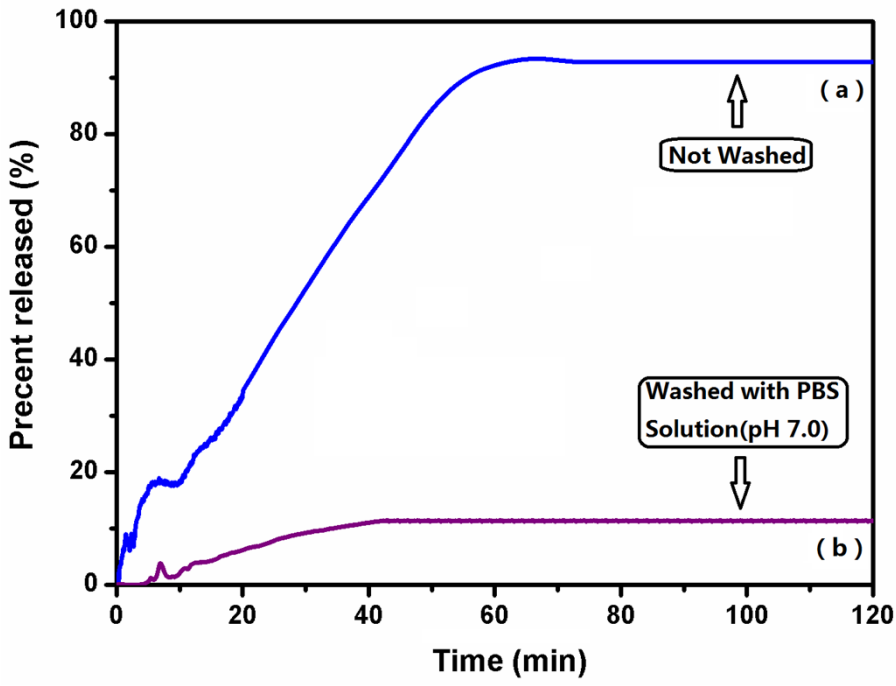

Figure S9. Release profiles of BTA-loaded MSNs **3** under pH 7.0 (a) not washed and (b) washed with PBS solution (pH 7.0)

In order to verify the effect of CB[6] macrocycles, the control experiments were performed. In the control experiments, BTA-loaded MSNs **3** were placed in the PBS solution (pH 7.0) and the UV/Vis absorbance intensity of BTA were monitored. From figure S9, the UV/Vis absorbance intensity increased rapidly under pH=7.0, which demonstrates that without CB[6] macrocycles gatekeepers, BTA molecules can diffuse out of MSNs **3** freely. After washed with PBS solution (pH=7.0), it can be easily seen that during the washing process the majority of BTA molecules were flowed out due to the lack of the protection of gatekeepers. Compared with the controlled-release results of MSNPs 1, it is undoubtedly that CB[6] macrocycles act as gatekeepers to regulate the flow of BTA molecules.