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Mono-benzimidazole functionalized β-cyclodextrins as supramolecular nanovalves for pH-triggered release of *p*-coumaric acid†

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The self-complexation of mono-benzimidazole functionalized β-cyclodextrins was investigated. The unique molecular structure employed as supramolecular nanovalves were installed on the external surface of mesoporous silica to assemble mechanized silica nanoparticles, which showed pH-triggered release property.

Nanovalves, as an important category of molecular machines, are capable of achieving effectiveness of valves at molecular dimensions and precisely controlling the flow of cargo molecules.[1] Nanovalves reversibly transform from "open" states to "closed" states by changing their molecular configurations or chemical bonding models, which results from environmental stimuli such as pH, temperature, redox, enzyme, and light.[2]

In recent decades, with the in-depth research of supramolecular chemistry, mechanically interlocked molecules, including bistable [2]catenanes, bistable [2]rotaxanes as well as pseudorotaxanes, are employed as supramolecular nanovalves to construct mechanized silica nanoparticles (MSNPs) and are responsible for accomplishing controlled release tasks, relying on the fact that the strength of non-covalent interactions between the two key components of nanovalves, stalks and macrocycles can be tuned by external forces.[3] Compared with the other traditional types of stimuli-responsive polymeric nanovalves, the MSNPs based on mesoporous silica nanoparticles (MSNs) as scaffolds have their own merits, such as tunable particle size, high loading capacity, negligible premature cargo leakage, sustainable release property, and show advantages in tumor-targeted drug delivery and smart anticorrosion coatings.[4] Unlike molecular nanovalves with the attribute of reversibility, supramolecular nanovalves are mostly irreversible upon activation, especially for [2]pseudorotaxanes. After the release processes, the macrocycles, such as cyclodextrin, cucurbituril, pillararenes, *etc.*, dethread from functional stalks, and the integrated structure of [2]pseudorotaxanes are destroyed.[5] In comparison, bistable [2]rotaxanes are more adaptable to be used as nanovalves. The reciprocating motions of macrocycles between two recognition sites upon stimuli execute the mission of "release–stop–release".[6] However, their preparations usually require multiple steps and are time-consuming.

The mono-functionalized macrocycles, mainly concentrating in cyclodextrin and pillararene, due to their easily decorated structures exhibit a distinctive self-complexation property in dilute solution.[7] To our knowledge, there have been no reports on the usage of self-complexation structure as supramolecular nanovalves so far. Herein, we envisage and prepare the mono-benzimidazole functionalized β-cyclodextrins by the introduction of 1*H*-benzimidazole (BZI) group at the secondary side of β-cyclodextrin (β-CD) *via* 1,3-dipolar cycloaddition reaction (Scheme 1A). Then, the mono-BZI functionalized β-CD as supramolecular nanovalves were installed on the surface of MSNs to assemble MSNPs **1**. In the neutral solution, BZI groups enter into the cavity of β-CD, forming self-complexation structure and blocking the diffusion channels. While in the acidic solution, BZI groups are protonated, the binding constant between BZI and β-CD decreases dramatically,[8] which results in the ejection of BZI groups. Then, the nanovalves are opened and cargo molecules can diffuse out through the cavities of β-CD (Scheme 1B). Benefiting from the self-switched characteristic, the hosts "β-CD" and the guests "BZI" are linked by triazole rings, which guarantees good probability for the reversibility of nanovalves and the reutilization of MSNPs **1**.

For the construction of mono-BZI functionalized β-CD, we decided to carry out the synthesis of mono-2-*O*-{1-(1*H*-benzoimidazol-2-ylmethyl)-1*H*-[1,2,3] triazol-4-ylmethyl}-β-cyclodextrin (compound **3**, Scheme 1A). First, we synthesized mono-2-*O*-propargyl-β-cyclodextrin (compound **1**) by a facile route. Taking advantage of terminal alkyne group linked to the O2 position, Cu(I)-catalyzed azide–alkyne Huisgen cycloaddition reactions of compound **1** was performed with 2-(azidomethyl)-1*H*-benzimidazole (compound **2**), which was obtained *via* nucleophilic substitution by using sodium azido solution to afford the click product compound **3** (the detailed synthesis process can be seen in ESI,† Schemes S1–S3, Fig. S1–S3). The self-complexation behaviour of

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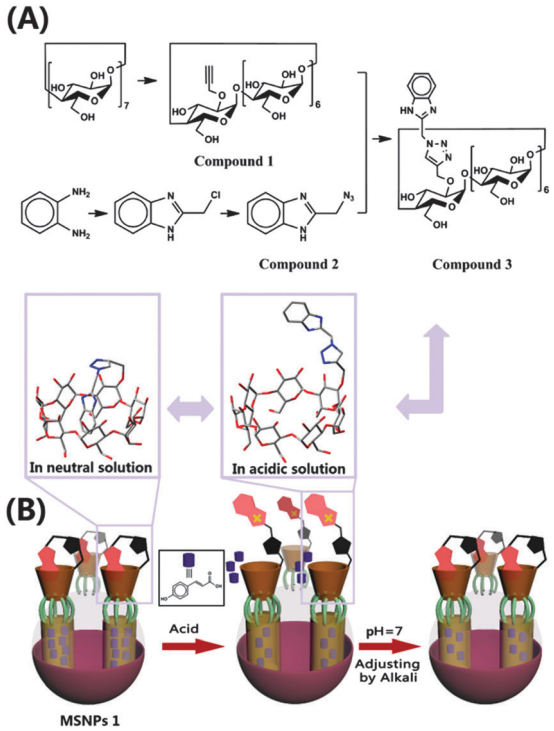

Scheme 1 (A) Synthesis route of compound **3**; (B) working mechanism of MSNPs **1**.

compound **3** was first investigated by ^{1}H NMR spectroscopy. The concentration of compound **3** was below 0.1 mM to avoid intermolecular self-assembly.[7] The ^{1}H NMR spectrum of compound **3** in D_2O is shown in Fig. 1A. Upon the addition of 1 equiv. of DCl (Fig. 1B), the peaks for BZI (H_a, H_b) displayed substantial downfield shifts ($\Delta\delta_a$ = 0.20, $\Delta\delta_b$ = 0.37 ppm) and a strong spectral simplification of the β-CD region was observed. After adjusting the pH to neutral using NaOD, the signals of H_a, H_b as well as H-3 and H-5 (marked in Scheme S1, ESI†), which are located inside the cavity of β-CD shifted remarkably upfield. The cyclic process shown in Fig. 1 indicates the self-inclusion of the BZI moiety into the hydrophobic β-CD cavity in neutral solution, and the dethreading occurs under

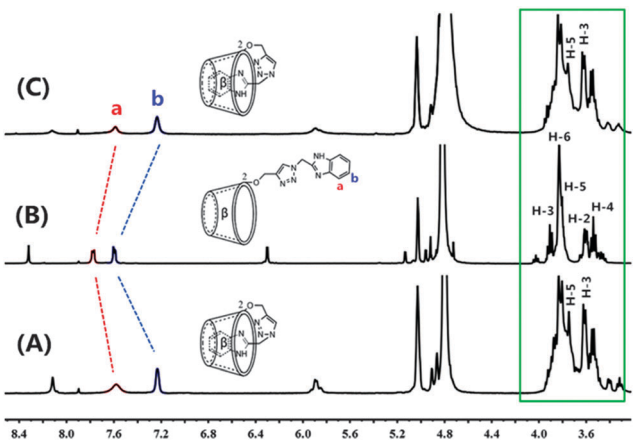

Fig. 1 ^{1}H NMR spectra of compound **3** in D_2O: (A) at 25 °C; (B) adding 1.0 equiv. DCl; (C) adding 1.0 equiv. NaOD.

acidic environment. The pH-dependent spatial conformation of compound **3** can be further confirmed by 2D ROESY (Fig. S4, ESI†). From 2D ROESY analysis, ROE correlations were observed between the protons of BZI and the protons of β-CD at neutral pH and disappeared in acidic condition. Further evidence for supporting switchable action was obtained by UV-vis measurements (Fig. S5, ESI†).

MCM-41-type MSNs with an average diameter of 150 nm, specific surface area of 1000.82 $m^2\ g^{-1}$ and pore size of 2.29 nm were synthesized according to a previously reported method (Fig. S6, ESI†).[9] The hexagonally arranged pores of the MSNs, which will accommodate cargo molecules were analysed using transmission electron microscopy (TEM) and small-angle X-ray diffraction (SA-XRD). The synthesis route of MSNPs **1** is shown in Scheme S6 (ESI†). Initially, the MSNs used as inorganic scaffold were reacted with (3-aminopropyl) trimethoxysilane (APTES) to obtain MSNs–NH_2. Subsequently, to facilitate supramolecular nanovalves (compound **3**) to attach the MSNs–NH_2, heptakis (6-deoxy-6-iodo) modified compound **3** was prepared (compound **4**, synthesis process is given in ESI†). Next, compound **4** was covalently anchored onto the external surface of MSNs through nucleophilic substitution reaction to afford MSNs–CD–BZI. Finally, the adsorption of *p*-coumaric acid as cargo molecules and the closure of supramolecular nanovalves were accomplished by precisely adjusting the pH of the solution, and then MSNPs **1** were assembled.

FTIR spectra (Fig. S7, ESI†) were used to characterize the two-steps of the functionalization process of MSNs. Compared with the Si–O–Si stretching (1080 cm^{-1}), Si–OH stretching (3434 cm^{-1}) and bending vibrations (1621 cm^{-1}) of bare MSNs, new peaks at 2933 and 2856 cm^{-1} in the MSNs–NH_2 were due to the asymmetric and symmetric C–H stretching vibrations, respectively, and a weak peak at 3078 cm^{-1} was ascribed to the N–H stretching vibration. Upon the functionalization of compound **4**, the appearance of peaks at 1539, 1452 and 1386 cm^{-1} are assigned to the characteristic benzene rings, C=N stretching and C–N stretching vibration in BZI rings, respectively, which indicates the successful attachment of supramolecular nanovalves. The grafting of different functional groups onto the surface of MSNs was further confirmed by the solid-state ^{13}C and ^{29}Si CP/MAS NMR spectra, as depicted in Fig. 2. The NMR spectrum of MSNs–NH_2 shows three resonance signals at about 38, 18 and 5 ppm, which were assigned to characteristic carbon peaks of i, j, k on 3-aminopropyl groups. In the NMR spectrum of MSNs–CD–BZI, apart from the peaks of i′, j′, k′ carbons, a series of new signals were clearly observed due to the resonances of nanovalves. The additional resonances were divided into two regions: (i) the signals at 97, 78, 68 and 52 ppm correspond to the carbon peaks of C_1, C_4, $C_{2/3/5}$ and C_6 on the β-CD, respectively; (ii) the signals at the range of 158–125 ppm were attributed to the characteristic carbon peaks on BZI groups. In addition, the ^{29}Si spectrum of MSNs–CD–BZI revealed bulk silicon peaks (Q region) around −124 to −90 ppm and T type signals (T region) at about −84 to −61 ppm, confirming functionalized silica resonances. The zeta potentials for MSNs, MSNs–NH_2 and MSNs–CD–BZI at pH 7.0 were −22.5, +7.5 and +1.9 mV, respectively. This changing tendency also confirms that the two-step functionalization proceeded smoothly.

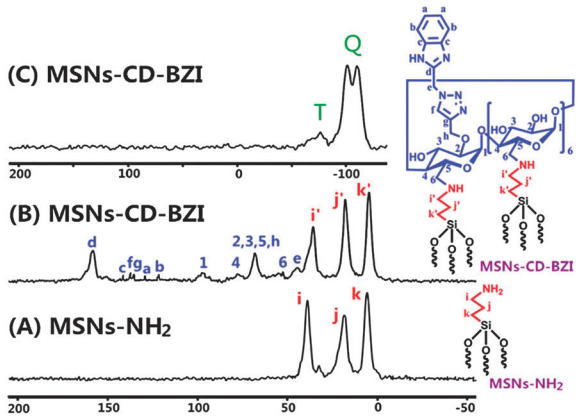

Fig. 2 ^{13}C CP-MAS solid-state NMR spectra of (A) MSNs–NH_2; (B) MSNs–CD–BZI and (C) ^{29}Si CP-MAS NMR spectrum of MSN–CD–BZI.

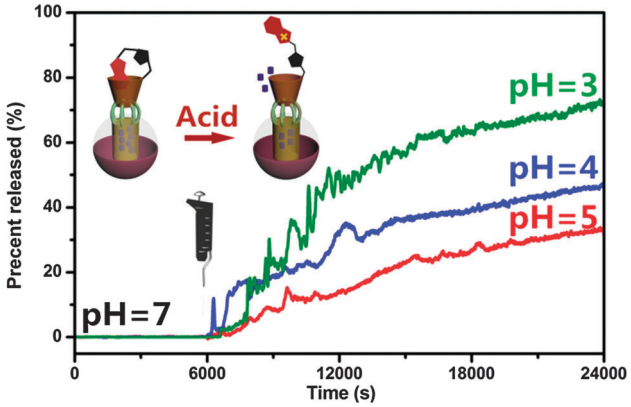

Fig. 3 Release profiles of MSNPs **1** at different pH values.

The grafting contents of APTES and compound **3** onto MSNs were determined to be approximately 1.457 and 0.15 mmol g^{-1} MSNs, respectively, by thermogravimetric analysis (Fig. S8, ESI†). Based on the calculation method proposed by Zhao *et al.*,[10] one mesopore is capped with one supramolecular nanovalve under ideal conditions by seven C–N bonds, as illustrated in Scheme 1B. Therefore, the roughly estimated grafting efficiency appears satisfactory. The MSNs–NH_2 and MSNs–CD–BZI exhibited a certain decrease in the specific surface area, pore volume, as well as pore size, which can be attributed to the partial occupation of pore channel of the stalks (Fig. S9 and Table S2, ESI†). However, through careful analysis of SA-XRD patterns (Fig. S10, ESI†) and TEM images (Fig, S11, ESI†), it can be concluded that after functionalization, MSNs–CD–BZI maintain the hexagonal mesoporous structure, which is beneficial for the subsequent adsorption of cargo molecules.

To facilitate the diffusion of *p*-coumaric acid into MSNs–CD–BZI, the loading process was performed in acidic solution at 60 °C. Acidic environment ensures that the nanovalves are in the open state, and the thermal effect accelerates the self-decomplexation rate enhancing the adsorption capacity. After 48 h, the pH of the solution was gradually adjusted to neutral and the solution was simultaneously cooled to room temperature, and then MSNPs **1** were collected for release experiments. To investigate the pH-dependent gating effect, MSNPs **1** were placed at various pH values, and the release amount of *p*-coumaric acid was determined by measuring fluorescence intensity at 439 nm (λ_{exc} = 351 nm), which was converted to the corresponding concentration according to the standard curve generated automatically by spectrophotometer. As shown in Fig. 3, under neutral pH, almost no release was observed because the pore outlets were blocked by nanovalves. Upon turning the pH to acidic, a sustained-release was detected with approximately 32%, 47% and 76% of the cargo molecules released within 400 min at pH 5.0, 4.0 and 3.0, respectively. These imply that the self-decomplexation between BZI and β-CD under acidic solution and the nanovalves were open to *p*-coumaric acid. The release rate was significantly accelerated on decreasing pH values. When the pH of the solution was below 6.0, the protonation of the BZI moiety (pK_a value of 5.68) occurs, resulting in significant decrease in binding affinity, dissociation of BZI from β-CD and the destruction of the originally stable self-complexation structure (under pH 7.0).[11] The stronger the acidity is, the higher is the degree of the protonation of BZI groups, and the more entrances are open to release cargo molecules. A series of control experiments were performed to corroborate the switching function of supramolecular nanovalves. As shown in Fig. S12 (ESI†), MSNs-CD (MSNs functionalized with heptakis (6-deoxy-6-iodo)-β-CD and no BZI groups linked through O_2 position) did not demonstrate pH-controlled release property, confirming the central role of the mono-BZI functionalized β-CD in the working mechanism.

Furthermore, according to our design, the self-complexation/decomplexation between β-CD and BZI makes the supramolecular nanovalves operate reversibly. To demonstrate their reversibility, MSNPs **1** were immersed in a solution, in which the pH was carefully adjusted as the route of "neutral–acidic–neutral–acidic", and the staged release profile is shown in Fig. 4. As expected, the states of the nanovalves depended on pH values, and the release of cargo molecules was triggered by the addition of acid and shut off in the neutral solution. It is worthwhile to note that when pH changed from acidic to neutral, the MSNPs **1** took short time to achieve equilibrium and the nanovalves quickly closed again to inhibit the flow out of cargo molecules.

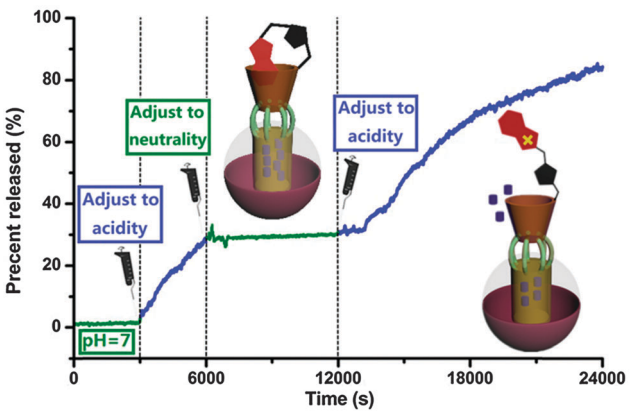

Fig. 4 Staged release profile of MSNPs **1**.

In summary, we have successfully assembled MSNPs **1** based on the mono-BZI functionalized β-CD as supramolecular nanovalves, which show a pH-triggered release character and maintain the reversibility of the nanovalves. Studies are ongoing to investigate their performance of cellular targeted drug delivery and construct the multi-stimuli responsive systems on the basis of self-complexation systems.

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