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Mesoporous silica nanoparticles (MSNs) have been surface-functionalized with choline moieties encircled by sulfonatocalix[4]arenes. Two enzyme cleavable sites are incorporated in the stalks for specific enzymes to regulate the release of loaded cargos from MSNs. These gated materials show a slow enzymatic response and proven orthogonality.

Mesoporous silica-based stimulus-responsive nanovalves have been paid great attention owing to their excellent performance in drug delivery, sensing, imaging, and so on.5 Compared with traditional controlled release systems, they show negligible premature cargo release before activation upon fine-tuning the valve tightness so that drug molecules can be protected and transported to target cells or tissues accurately, which not only improves the therapeutic efficacy of drugs, but also reduces the adverse side effects. As the solid support of molecular or supramolecular nanovalves, mesoporous silica nanoparticles (MSNs) are recognized as smart nanocontainers for the biapplications of drug delivery, imaging and tumor therapy, attributing to the large surface areas, suitable pore volumes, tunable pore sizes, chemically modifiable surfaces, and good biocompatibility.2 To better control the loaded therapeutic compounds, many materials are crafted on the surface of MSNs,3 including organic molecules,4 inorganic nanoparticles,5 polymers6 and molecular machines.7 Significantly, a series of external stimuli have been employed to operate the nanovalves for the entrapment and release of cargos from the pores of MSNs, such as pH,4 light,3,5,6 heat,3,5,6 redox,7 and enzymes.4b,7c

Early in 2003, Fujiwara and his co-workers8 designed the first stimuli-responsive gated MSN system with coumarin molecules modified on the surfaces of MSNs, where the release of cargos was regulated by light activation. However, premature cargo release remained a major unresolved problem. In 2004, the first supramolecular actuator on nanoparticles (NPs)7a was successfully synthesized by Stoddart, Zink, and others, who managed to combine traditional MCM-41 NPs with molecular machines to result in the emergence of molecular and supramolecular nanovalves. Since then, a series of nanovalve systems were constructed, where macrocyclic receptors and stalk components constitute [2]rotaxanes or [2]pseudorotaxanes around the pores of MSNs in the following designs. Major macrocyclic rings have been used in the construction of nanovalve systems, such as CBPQT4+,7a crown ethers,9 cyclodextrins,7c cucurbit[n]urils7 and pillar[n]arenes.2b However, calix[n]arenes as the third generation of macrocyclic receptors in supramolecular chemistry,8 which are made up of phenol units linked by methylene bridges, have not yet been employed in the field of stimulus-responsive nanovalves for controlled cargo release, although they have been incorporated with mesoporous silica materials in the literature.10,11 Among the many different analogues of calix[n]arenes, watersoluble sulfonato-calix[n]arenes (SC[n]As) have been reported to be biocompatible and possess many advantages in molecular recognition and self-assembly and applications in various fields of separation, materials science, self-assembly membranes and super-amphiphiles.11 We envision that sulfonatocalix[4]arene (SC[4]A) can be a good candidate to be applied in nanovalve systems for controlled anti-cancer drug release.

Herein, SC[4]A-based biocompatible supramolecular nanovalves operable by multiple external stimuli, triple herein, i.e., enzyme, pH variation, and competitive binding, are shown in Fig. 1. SC[4]A can form stable 1:1 complexes with choline and its derivatives, e.g., the binding constant for the 1:1 inclusion complex of SC[4]A and choline is ca. $10^3$ M$^{-1}$.11 Therefore, two choline derivatives with different structures and lengths (Fig. 1) were modified onto the pore orifices of MSNs via ester linkage or urea bond as enzyme cleavage sites in the

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stall components of nanovalves. It is a desired choice to use enzymes to control the opening of the pores of MSNs, because (i) most of the enzymes show the highest specificity, accuracy and efficiency for the improvement of the targeted release and release effects; (ii) many enzyme activations can occur under mild conditions showing the lowest side effects to the human body during nanovalue activation; and (iii) enzyme activity is sensitive to the surroundings, e.g., pH, temperature, and the relative inhibitor, which makes it easy for adaptation. More significantly, the enzyme-responsive nanovalue systems can be also fine-designed to be operable by adding competitive binding agents or changing pH values to realize multi-responsiveness. Although supramolecular motifs have been used before for controlling the release of MSNs, the route shown in this paper is quite novel due to its clear enzymatic response and proven orthogonality. As a proof-of-concept, luminescent rhodamine B (RhB) is loaded in the pores of MSNs as model drugs and UV-vis spectroscopy is employed to monitor the controlled cargo release upon nanovalue operation.

The fabrication of the SC[4] A-based nanovalue systems are depicted in Fig. 1 and the ESI†. The MSNs used in the present study were prepared using a template-directed sol-gel method. Briefly, cetyltrimethylammonium bromide (CTAB) was used as the template and tetraethylorthosilicate (TEOS) as the silicon source under weak basic conditions to form regular pore structures, followed by the removal of the template through extraction of the MSNs with hot acidified MeOH. The organic functionalization of traditional MSNs (MSN-OH) was carried out via a co-condensation synthesis method or post-functionalization to obtain carboxylic MSNs (MSN-COOH) and choline-modified silica nanoparticles (MSN-C1 with ester-linked stalks and MSN-C2 with urea-linked stalks). Then, the negatively charged SC[4] A macrocycles, synthesized using a modified procedure based on literature reports (see the ESI†), were introduced to encircle the choline stalks on the surfaces of MCM-41 NPs via host-guest complexation to form [2]pseudorotaxanes as the movable/cleavable elements of the nanovales.

The morphological study of MSN-OH and MSN-COOH was done using scanning electron microscopy (SEM) (Fig. 2), which shows that both MSN-OH and MSN-COOH are well mono-dispersed and homogeneous spherical NPs, with an average size of ca. 160 nm in diameter. Transmission electron microscopy (TEM) reveals the existence of ordered 2D hexagonal arrays of cylindrical nanopores (ca. 2.8 nm in diameter for MSN-C1, ca. 2.4 nm for MSN-C2) of the MCM-41 NPs before surface functionalization. But, after cargo loading and SC[4] A capping, the MSNs exhibit less clear pore structures due to the existence of cargos contained in the pores, and SC[4] As and choline stalks on the orifices of the pores. This feature was further confirmed by the small-angle powder X-ray diffraction (XRD) pattern displayed by the freshly obtained MSNs. The intensities of the XRD peaks (Fig. 3a and Fig. S3, ESI†) become lower after the covalent modification of the MCM-41 NPs and decrease even more sharply after cargo loading and SC[4] A capping of the choline stalks. In addition, the surface areas and pore widths of the resulting MSNs were measured by nitrogen adsorption–desorption isotherms (Fig. 3b, Fig. S6–S10, ESI†). Clearly, before loading with RhB and capping with SC[4] A these samples exhibit characteristic Type IV Brunauer-Emmett-Teller (BET) adsorption isotherms, consistent with the existence of cylindrical nanopores. A pronounced step is exhibited at a relative pressure ranging from 0.2 to 0.7 [P/P0] prior to RhB-loading and SC[4] A-capping of the MCM-41 NPs due to the capillary condensation of nitrogen inside the MSN nanopores. A narrow pore size distribution is observed in accordance with the steep condensation step. The properties of MSN-OH, MSN-COOH, the choline derivative-modified MSNs and the RhB-loaded, SC[4] A-capped MSNs (MSN-C1 and MSN-C2) are summarized in Tables S3 and S4 (ESI†). The successful functionalization of MSNs was further validated using Fourier transform infrared spectroscopy (FT-IR) (see the ESI†). Surface coverage of functionalities was calculated using elemental analysis (see the ESI†).

We first set out to employ the nanovalue systems as an enzyme-responsive model to investigate the cargo release property. A sample of the RhB-loaded, SC[4] A-capped MSNs (2 mg) was placed in the dialysis bag in a cuvette (Fig. S1, ESI†) and PBS buffer (pH = 7.4 or 8, 3 mL) was slowly added. Then a buffer solution containing enzymes (Final C_{enzyme} = 0.3 mg mL^{-1}, pH 7.4 or 8) was added with gentle stirring. Release of RhB from the nanopores of the MSNs was monitored using UV-vis absorption spectroscopy. As a control experiment, the release of RhB from RhB-loaded, SC[4] A-capped MSN-C1 and MSN-C2 are also examined under the same conditions but in the absence of enzymes or in the presence of deactivated enzymes. The bottom lines show (Fig. 4) that there is a limited premature release which can be further suppressed by adding more SC[4] As to the solution (see the ESI†). In addition, we also activated these MSNs by changing pH or by adding competitive binding agent, i.e., ethanediamine, showing faster release rates as compared with enzyme activations due to the ease of interaction between the nanovales and the smaller activation agents (Fig. 4).

It is worth noting that esterase can only activate the RhB-loaded, SC[4] A-capped MSN-C1 with an ester linkage in the stalk component of the nanovales and urease can only activate the RhB-loaded, SC[4] A-capped MSN-C2 with a urea linkage in the stalk component. Herein, an unmatched enzyme is added into the solution with MSN
materials under the optimum pH. Fig. 5 shows that only a limited release can be observed with the addition of the unmatched enzyme, which is in sharp contrast to the matched activation. Therefore, another advantage of these nanovalve systems that has been observed is the high specificity and accuracy, namely orthogonality. Moreover, cargo release upon enzyme addition for different pH values is conducted to rule out the influence of surrounding media (Fig. S2, ESI†). The results show that a good release can only be obtained at the optimum pH of the employed enzymes. It is thus clear that having a comfortable surrounding is necessary for the enzyme to show the maximum activation power.

In summary, two types of biocompatible nanowalls based on MSNs (MSN-C1 and MSN-C2) have been produced through the introduction of biocompatible stalks, containing choline derivatives and enzyme cleavage sites, onto the surface of mesostructured silica, followed by cargo loading via diffusion and calix[4]arene capping via host–guest complexation. These versatile systems are capable of entrapment and controlled release of cargo molecules in response to three different types of external stimuli, i.e., enzyme, pH variation and adding competitive binding agent. One type of functionalized MSN has been used to prepare two different nanowall systems, one with an ester-linked tether and the other with a urea-linked tether, to show a clear enzymatic response and proven orthogonality. Using UV–vis spectroscopy, we have demonstrated the ability of esterase to selectively activate the ester-linked version of nanowalls (MSN-C1), and urease to selectively activate the urea-linked version of nanowalls (MSN-C2), while the unmatched tether-linked system is left intact.

Compared with other control methods, enzyme activation has shown several advantages including the highest enzymatic specificity and efficiency, mild activation conditions, ease of adaptation, and sustained release profiles.

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Notes and references


