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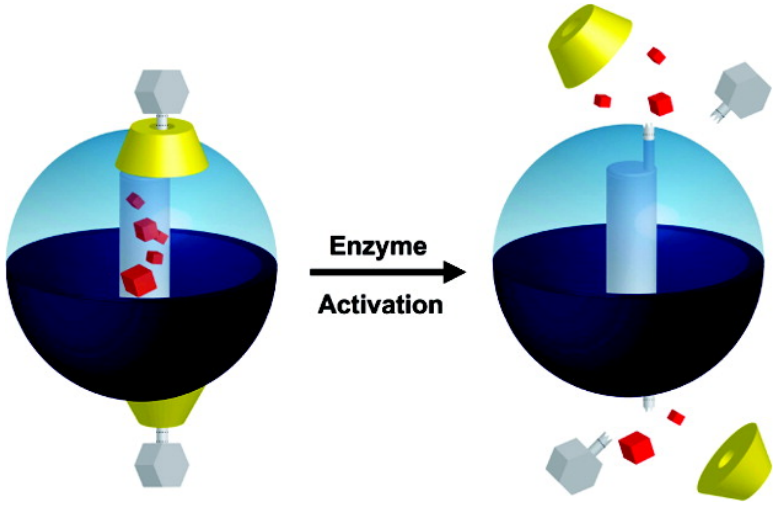

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Enzyme-Responsive Snap-Top Covered Silica Nanocontainers

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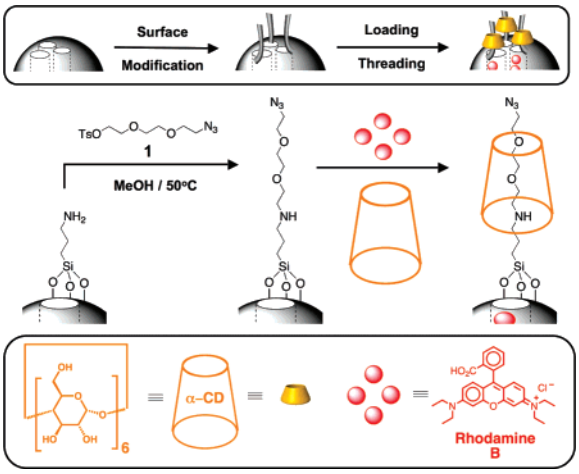
Mesoporous silica nanoparticles coated with molecular valves hold the promise to encapsulate a payload of therapeutic compounds, to transport them to specific locations in the body, and to release them in response to either external or cellular stimuli. Sequestering drug molecules serves the dual purpose of protecting the payload from enzymatic degradation, while reducing the undesired side-effects associated with many drugs. Although these benefits are common to pro-drug strategies,[1] the nanoparticle-supported nanovalve system does not require covalent modification of the therapeutic compounds and allows for the release of many drug molecules upon each stimulus event.[2] Recently, it was demonstrated that mesoporous silica nanoparticles, *not* modified with molecular machinery, can deliver the water-insoluble drug camptothecin into human pancreatic cancer cells with very high efficiency.[3] For more sophisticated drug delivery applications, the ability to functionalize[4] nanoparticles with nanovalves and other controlled-release mechanisms has become an area of widespread interest.[5] Previously, we have demonstrated the operation of molecular and supramolecular valves in nonbiologically relevant contexts using redox,[4a,b,e] pH,[4c] competitive binding,[4d] and light[4f] as actuators. Other controlled release systems include photoresponsive azobenzene-based nanoimpellers,[5d] chemically removable CdS nanoparticle caps,[5b,e] and reversible photodimerization of tethered coumarins.[5a]

Herein, we report the design, synthesis, and operation of a novel, biocompatible controlled-release motif we call snap-top covered silica nanocontainers (SCSNs). Silica nanoparticles (~400 nm in diameter) that contain hexagonally arranged pores (~2 nm diameter) function as both the snap-top supports and as containers for guest molecules. The porous mesostructure[6] is templated by cetyltrimethylammonium bromide (CTAB) surfactants, and particle synthesis is accomplished using a base-catalyzed sol−gel procedure.[7] Methods for derivatizing silica are well-known[8] and are used here to functionalize the nanoparticle surfaces with the snap-top machinery. In general, a snap-top consists of a [2]rotaxane tethered to the surface of a nanoparticle in which an α-cyclodextrin (α-CD) torus encircles a polyethylene glycol thread and is held in place by a cleavable stopper. When closed, the snap-top contains guest molecules stored within the pores, but releases the guests following cleavage of the stopper and dethreading of the torus. On the basis of the design of the stopper, we conceive that a multitude of stimuli could be exploited to activate snap-top systems. The specific snap-top system we describe here releases encapsulated cargo molecules following enzyme-mediated hydrolysis.

A divergent approach has been taken in the design and synthesis of SCSNs in which the use of a single versatile snap-top precursor enables the preparation of multiple systems that are ultimately highly specific and differentiated in their function. In the divergent design, a snap-top precursor consisting of unstoppered [2]pseudorotaxanes serves as a foundation from which various snap-top systems can be created depending on the specific stopper that is attached. The synthesis of the snap-top precursor is carried out in a stepwise fashion from the nanoparticle surface outward (Scheme 1). The mesoporous silica is first treated with aminopropyltriethoxysilane to achieve an amine-modified surface. The amine-functionalized material is then alkylated with a tri(ethylene glycol) monoazide monotosylate unit to give an azide-terminated surface. Cargo molecules are loaded into the nanopores by diffusion, and the loaded, azide-modified particles are then incubated with α-CD at 5 °C for 24 h. The α-CD tori thread onto the tri(ethylene glycol) chains at low temperature, effectively blocking the nanopores, while the azide function serves as a handle to attach a stoppering group. The stoppers are chemically attached to the snap-top precursors using the Cu(I)-catalyzed azide-alkyne cycloaddition,[9] a transformation chosen because of its remarkable functional group tolerance and high efficiency as well as our recent success in utilizing it for the preparation of interlocked compounds.[10]

Scheme 1. Synthesis of the SCSN Precursor



To test the viability of an enzyme-responsive snap-top motif, a system activated by porcine liver esterase (PLE)[11] was designed (Scheme 2). To prepare a PLE-responsive SCSN, a precursor loaded with luminescent cargo molecules (rhodamine B) was capped with the ester-linked adamantyl stopper **2a**. In this snap-top system, PLE catalyzes the hydrolysis of the adamantyl ester stopper, resulting in dethreading of the α-CD, and release of the cargo molecules from the pores. As a control, an SCSN was also prepared using the adamantyl amide analogue **2b**, which does not undergo hydrolysis by PLE. After the stoppering reactions, the dye-loaded silica particles were filtered and washed to remove nonspecifically adsorbed small molecule contaminants.

The successful functionalization of the nanoparticle surface was confirmed by FT-IR spectroscopy at various stages of loading and release (see Supporting Information). For the azide-modified nanoparticles, the peak at 3450 cm^{-1} is indicative of an N−H stretch, while a strong absorption between 1050 and 1300 cm^{-1} indicates the presence of different kinds of C−N bonds. The control amide snap-top system shows two distinctive absorption peaks for

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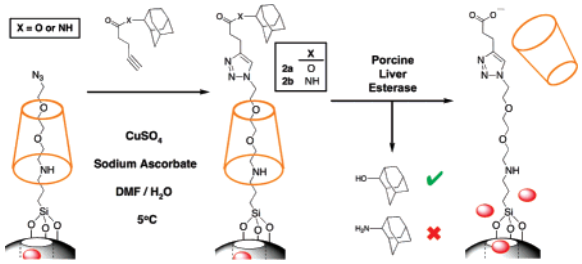
Scheme 2. Synthesis and Activation of Enzyme-Responsive Snap-Top System



the amide C═O group at 1650 and 1600 cm^{-1}. The ester-functionalized snap-top system shows instead the expected ester C═O stretch at 1731 cm^{-1} with pronounced C−H absorptions arising from the adamantyl group. In the spectra of the nanoparticles after guest release, the region around 3000 cm^{-1} is broad, a feature which is characteristic of the new carboxylic acid functionality while the C═O peak is still evident at 1731 cm^{-1} indicating some remaining ester functionalities on the surface of the nanoparticles.

The enzyme-triggered release of cargo molecules was monitored using luminescence spectroscopy. The dye-loaded, stoppered particles (15 mg) were placed in the corner of a cuvette before carefully adding HEPES buffer (50 mM, 12 mL, pH = 7.5). To open the snap-tops, a solution of PLE [0.12 mL, 10 mg/mL in 3.2 M $(NH_4)_2SO_4$] was carefully added while the solution was stirred. The emission of rhodamine B in the solution above the particles was measured as a function of time using a 514 nm probe beam (15 mW), both before and after addition of PLE (Figure 1).

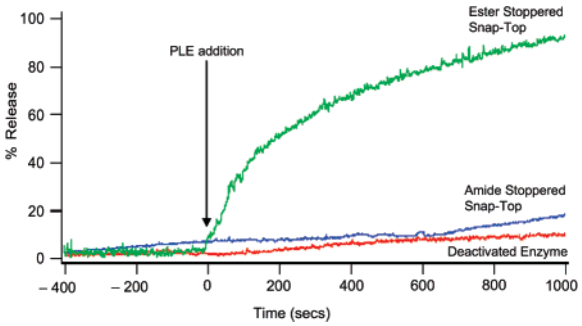


Figure 1. Controlled release of rhodamine B from ester (green) or amide (blue) stoppered snap-tops. The response of the ester-stoppered system to the deactivated enzyme (red) is also shown.

Prior to the addition of PLE, the emission intensity of rhodamine B is essentially constant, indicating that the dye remains trapped in the pores of the silica particles. The emission intensity begins to increase almost immediately following addition of PLE. The emission intensity asymptotically approaches its maximum value with a half-life of ~5 min. By contrast, no such increase in emission was observed for the amide-stoppered snap-top system. To further demonstrate that the enzyme is responsible for the release, it was denatured by heating at 50 °C for 30 min before addition to the ester-stoppered snap-tops. No release of dye was observed. Taken together, these results are consistent with the specific opening of the snap-tops as a result of the enzyme-mediated hydrolysis of the adamantyl ester stoppers.

To estimate the payload of molecules that are released by the snap-top system, the absorbance of the solution above the particles was measured before and after release. Using these data, it was calculated that for 15 mg of particles, 0.45 μmol (1.4 wt %) of rhodamine B is released.

Described herein is a versatile system that is capable of entrapment and controlled release of cargo molecules. We have used one snap-top precursor to prepare two different snap-top systems, one with an ester-linked stopper and the other with an amide-linked stopper. Using luminescence spectroscopy, we have demonstrated the ability of PLE to selectively activate the ester-linked snap-top system while the amide-linked system is left intact. The result of this work is a biocompatible controlled release system that exploits enzymatic specificity. Because of the wide range of stoppering units that could be attached to the SCSN precursor, a multitude of snap-top systems with differentiated modes of activation could be prepared with relative ease. In the future, the divergent synthetic approach that we have described will allow the snap-top motif to be very easily adapted to accommodate many different applications.

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Supporting Information Available: Experimental details, spectral characterization data of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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