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Snap-Top Nanocarriers

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ABSTRACT

An approach to the design and fabrication of mechanized mesoporous silica nanoparticles is demonstrated at the proof of principle level. It relies on the reductive cleavage of disulfide bonds within an integrated nanosystem, wherein surface-bound rotaxanes incorporate disulfide bonds in their stalks,- which are encircled by cucurbit[6]uril or α-cyclodextrin rings, until reductive chemistry is performed, resulting in the snapping of the stalks of the rotaxanes, leading to cargo release from the inside of the nanoparticles.

In recent years, a range of molecular and supramolecular nanovalves[1] have been attached covalently to the surfaces of mesoporous silica nanoparticles[2] in order to generate mechanized silica nanoparticles[3] (MSNPs) capable of the controlled release of cargos in the shape of small molecules, e.g., dyes and drugs. The MSNPs have been shown to exhibit this controlled release under the influence of pH changes,[4−6] competitive binding[5] conditions, redox activation,[1,7] and

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light-initiated[2b,8] stimuli, not to mention biological triggers.[9,10] In the so-called snap-top[10] version of molecular nanovalves, one of the stoppers of a rotaxane, the other one being the mesoporous nanoparticle itself, is cleaved enzymatically, allowing the ring component (gate) to escape from its stalk. The modular synthesis of these snap-top systems can lead to a high degree of variation in the separate components, i.e., the solid support, the linear stalk, the gate, and the bulky stopper, of the integrated system. The challenge now is to impart upon these "smart" MSNPs the ability to have them target[11] specific diseased cells during their action in the body and then, and only then, release the killer molecules.

Many current drug delivery systems incorporate disulfide linkages[12] that are reduced subsequently by glutathione from the cell cytosol.[13] This biochemistry permits such integrated systems to operate autonomously by utilizing the chemical processes that are already operating within cells to effect the release of drug payloads, thus eliminating the need for external stimuli. This disulfide bond provides an excellent cleavage handle for intracellular delivery because of the significantly higher concentration[14] of glutathione present within cells (~10 mM) compared with that in the bloodstream (~2 μM). Herein, we describe an integrated system that utilizes mesoporous MCM-41, functionalized with disulfide-containing [2]rotaxanes, to release their cargos selectively upon exposure to chemical reductants. While the snap-top nanoreactors presented in this communication are not the first to utilize the reductive cleavage of the disulfide bond in order to release cargo molecules from the interior of mesoporous silica nanoparticles,[12a,c] this particular integrated system is unique insofar as it employs a highly modular approach to the piecing together of the individual building blocks that extend outward from the surface of the MSNPs and thus are amenable rather easily to customization.

Various gates, for example, cucurbit[6]uril[15] (CB[6]) and α-cyclodextrin[16] (α-CD), can be employed (Scheme 1) in aqueous solutions for the controlled release of cargos using nanovalves as the key components of MSNPs. These two donut-shaped rings are both capable of acting as recognition tori during the template-directed syntheses[17] of mechanically interlocked molecules[18] (MIMs), including molecular switches and machines. Both macrocycles are soluble in H_2O and are biologically benign, making them ideal building blocks for incorporation into integrated systems for biological applications. Whereas CB[6] exhibits D_{6h} symmetry and consists of six glycoluril units, bridged one with another in a cycle array, linked by pairs of methylene groups,[15] α-CD is a cyclic oligosaccharide containing six α-1,4-linked D-glucopyranosyl residues and so has C_6 symmetry. Both of these gates are capable, in aqueous environments, of blocking the pores of the MSNPs, thus preventing the cargo from escaping from inside the nanoparticles until such times as the gates are released from the stalks by, for example, the reduction of disulfide bonds positioned along the stalks at the point of attachment of the stoppers. Cleave these bonds, and off come the stoppers, leaving the capping agents free to part company with the stalks and consequently release the cargo molecules.

Mesoporous silica has proven to be an ideal scaffold for a wide range of nanosystems, largely because of the fact that it is glass, which is indeed rigid, chemically inert, and optically transparent.[1−10,19−21] In this communication, we relate how a well-known sol−gel procedure[22] has been employed under basic conditions to obtain spherical mesoporous silica nanoparticles (see Supporting Information) with an average diameter of 100−200 nm, containing 2 nm

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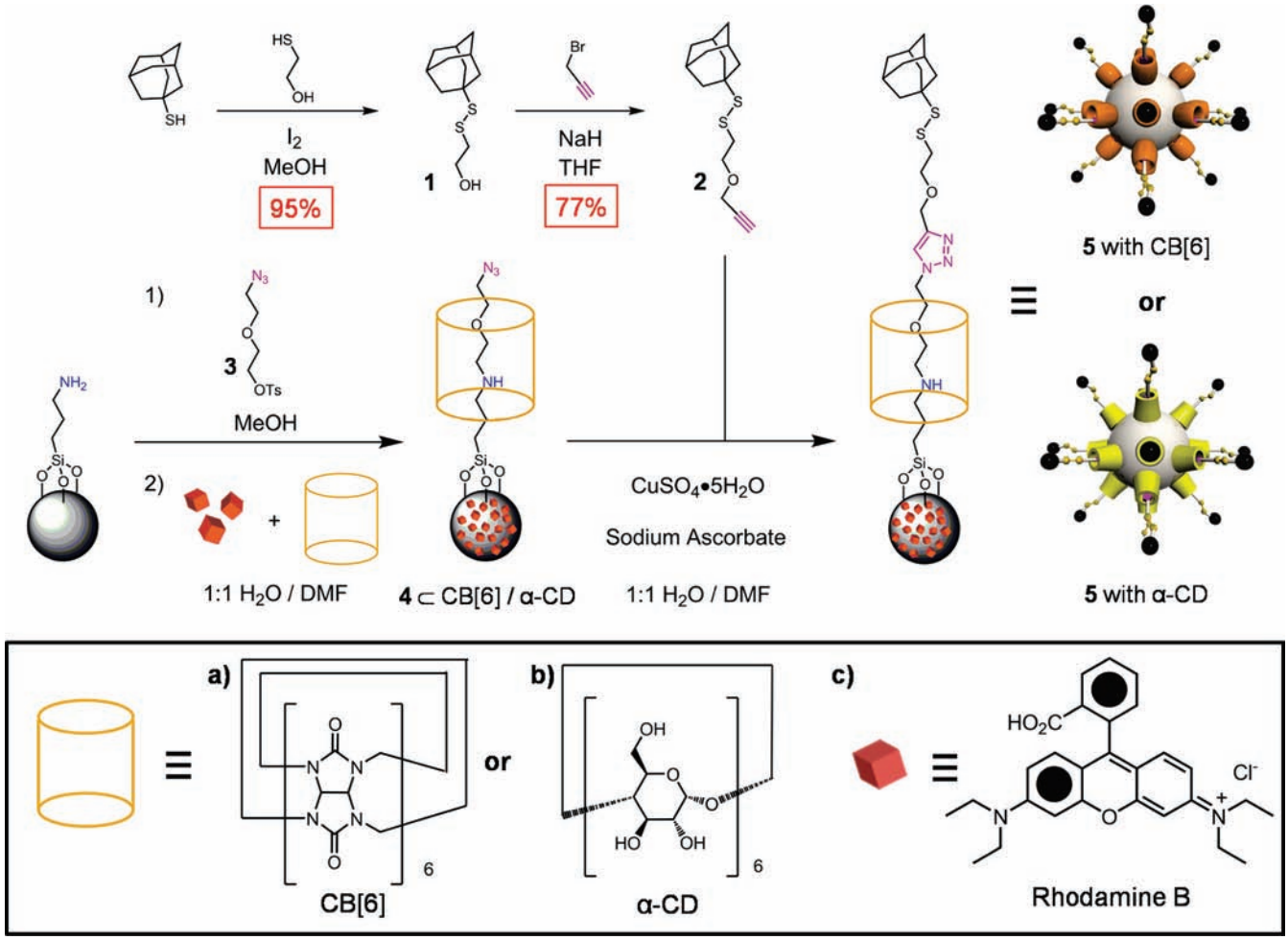
Scheme 1. Synthesis of the Alkyne-Terminated Stopper and Assembly of Disulfide-Based Snap-Top Nanocarriers[a]



[a] The nanocarriers studied in this report utilize mesoporous silica nanoparticles (MCM-41) as the delivery vehicle, CB[6] or α-CD as the capping agent (gate), and Rhodamine B as the cargo. (a) Structural formula of CB[6], represented graphically by pumpkin-colored barrels. (b) Structural formula of α-CD, represented graphically by a yellow lamp-shade cartoon. (c) Structural formula of Rhodamine B, represented graphically by rose-colored cubes.

diameter pores with a lattice spacing between them of about 4 nm. These dimensions were revealed by powder X-ray diffraction and scanning electron microscopy (see Supporting Information), in addition to transmission electron microscopy. Because of the organic−inorganic hybrid nature of these delivery vehicles, we cannot call on traditional characterization techniques such as ^{1}H NMR and ^{13}C NMR spectroscopies or mass spectrometry to assess the identity and purity of the completed nanocarriers.

A monolayer of [2]rotaxanes covering the entire surface of the spherical silica nanoparticles was engineered in a stepwise, divergent manner[6,10] using a surface-outward approach from mesoporous MCM-41. The bare nanoparticles were first of all functionalized with aminopropyltriethoxysilane monomers (Scheme 1), which were then reacted with the azide-terminated diethyleneglycol monotosylate **3**, followed by the addition of Rhodamine B and an excess of either CB[6] or α-CD to yield the complex **4**⊂CB[6] or **4**⊂α-CD. An excess of the gate, either CB[6] or α-CD, can be used in this procedure to ensure that all, or nearly all, of the stalks on the surface of the nanoparticles are threaded, and the pores of the nanoparticles are sufficiently well sealed. This synthetic protocol lends itself to the production of a considerable collection of snap-top nanovalves, since different lengths[7] of silane linkers and poly(ethylene glycol) stalks can be employed. Ideally, short rotaxane stalks must be capable of binding the gate and trapping the cargo molecules, e.g., Rhodamine B, within the cylindrical cavities of the mesoporous silica, thus avoiding premature leakage of the cargo. Following the self-assembly of the complexes **4**⊂CB[6] and **4**⊂α-CD, the azide groups at the ends of the stalks permit us to construct the monolayer of [2]rotaxanes by carrying out[23]Cu(I)-catalyzed 1,3-dipolar cycloadditions with the propargyl ether **2**, obtained (see Supporting Information) from the alcohol **1** where bulky stoppers in the shape

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of the adamantyl groups are attached by means of disulfide linkages.

The release of Rhodamine B, which was chosen because of its spectroscopic properties, from the dye-loaded MSNPs was monitored by luminescence spectroscopy as a function of time. For each of the snap-top MSNPs, **5** with CB[6] and **5** with α-CD, a small amount (5−10 mg) of the dye-loaded MSNPs was placed in the bottom of a cuvette and either distilled H_2O or TRIS buffer (pH 8.3) was introduced into the cuvette. In the case of **5** with CB[6], a large excess (50 mg) of dithiothreitol (DTT) was added to the cuvette along with distilled H_2O. Distilled H_2O or aqueous buffer solution were employed as the medium for the release experiments, a situation which anticipates the expectation that these nanocarriers will be biocompatible. The reason an excess of DTT is added to the cuvette is to ensure that all disulfide bonds on the surface of the MSNPs are cleaved. The fluorescence emission spectrum was recorded (Figure 1) periodically at a wavelength of 580 nm, with the excitation being provided at 530 nm before and after the addition of DTT. In the case of the other MSNPs, 2-mercaptoethanol (ME) (200 μL) was employed to reduce the disulfide bonds in **5** with α-CD. On this occasion, fluorescence spectroscopy was carried out (Figure 2) in the presence of a TRIS buffer (pH 8.3). The Rhodamine B cargo started to diffuse into solution immediately after the addition of ME. These MSNPs underwent controlled release of Rhodamine B in distilled H_2O as well (see Supporting Information), but the release of the dye was much slower than that observed in the buffered solution. In addition, **5** with α-CD operated as expected when DTT was used as the reductant in distilled H_2O (see Supporting Information). In the case of both MSNPs, the release of the dye could not be detected prior to the addition of DTT or ME, i.e., there was no detectable leakage. We hypothesize that the different release rates could be attributed to CB[6] having a lower affinity to the rotaxane stalk than α-CD. However, we do not seek in this communication to make any claims about the rates of release or kinetic data of these delivery systems.

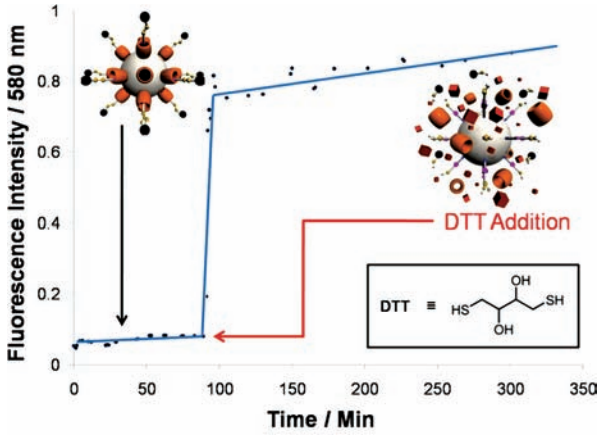

Figure 1. Release profile of snap-top nanocarrier **5** with CB[6], displaying the release of the Rhodamine B cargo upon exposure to DTT in distilled H_2O.

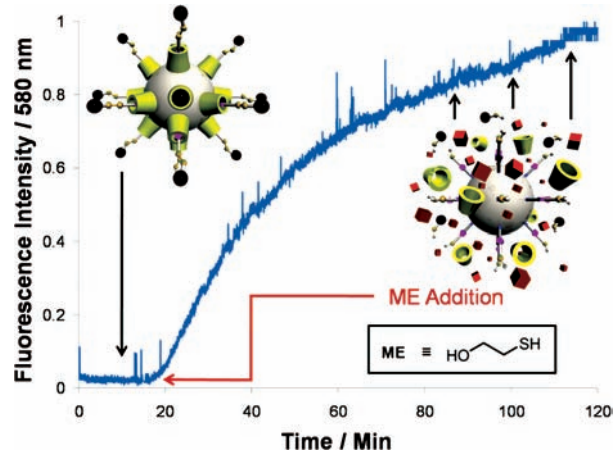

Figure 2. Release profile of snap-top nanocarrier **5** with α-CD, displaying the release of the Rhodamine B cargo upon exposure to ME in aqueous TRIS buffer at pH 8.3.

A range of MSNPs based on the snap-top principle[10] have been assembled such that they work under conditions of a chemically initiated, redox-controlled operation in aqueous solution. On account of the modular nature of the assembly of these snap-top carriers, each component of the integrated system can be tailored to install targeting features and containment of small drug molecules and/or imaging agents. Because of the integrated systems' modularity and tunability, in addition to being relatively simple to assemble, the snap-top carriers promise to be useful for a range of nonbiological as well as biological applications. The ability of these carefully crafted nanocarriers to discharge their cargos in aqueous media, in addition to releasing only nonreactive byproducts during the release event, confirms their biocompatibility and augurs well for them finding a passage into living cells.[24]

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Supporting Information Available: Description of the synthesis, characterization, and methods; additional release profiles and control studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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(24) This communication is dedicated to the memory of Professor Victor S.-Y. Lin, see: Buriak, J. M. *ACS Nano* **2010**, *4*, 2973–2974.