Nanotechnology

DOI: 10.1002/anie.201107960

Stimulated Release of Size-Selected Cargos in Succession from Mesoporous Silica Nanoparticles**

Cheng Wang, Zongxi Li, Dennis Cao, Yan-Li Zhao, Justin W. Gaines, O. Altan Bozdemir, Michael W. Ambrogio, Marco Frasconi, Youssry Y. Botros, Jeffrey I. Zink, and J. Fraser Stoddart**

Combination drug therapy,[1] a regimen in which multiple drugs with different therapeutic outcomes are used in parallel or in sequence, has become one of the dominant strategies in the clinical treatment of HIV/AIDS,[2] diabetes,[3] and cancer.[4] In cancer therapy, for example, the U.S. Food and Drug Administration (FDA) approved the use in 2006 of Avastin in combination with Carboplatin and Paclitaxel for the initial systemic treatment of patients with lung cancer. Unlike monotherapy, combination therapy maximizes therapeutic efficacy against individual targets and is more likely to overcome drug resistance, while increasing the odds of a positive prognosis and reducing harmful side effects.

Drug delivery systems, which administer medically active compounds to diseased cells in a targeted and controlled manner,[5] have gained much attention in the past couple of decades. While polymers,[6] dendrimers,[7] micelles,[8] vesicles,[9] and nanoparticles[10] have all been investigated for their use as possible drug delivery systems, most systems provide either delivery of a single drug or the simultaneous delivery[11] of multiple drugs. Using these systems, however, it is difficult to control[12] the administration order, timing, and dosage of each individual drug in a comprehensive manner. While it is possible to deliver a cocktail of drugs using several different co-administered drug delivery systems, this protocol has disadvantages. For example, it is not easy to expose several co-administered drug delivery systems to the same target at the right time, while also controlling the dosage rates and ratios of each individual drug. In order to administer chemotherapeutic combinations and produce synergistic actions, well-organized multidrug release systems, which can provide combination therapies by controlling the release behavior of each drug individually, need to be invented.

Mesoporous silica nanoparticles (MSNs) have attracted widespread interest[13] in the past decade for use in integrated functional systems. They have large surface exteriors and porous interiors that can be harnessed as reservoirs for small-molecule-drug storage. These MSNs are nontoxic to cells and can undergo cellular uptake[14] into acidic lysosomes by endocytosis when they are 100–200 nm in diameter, thus making them a popular candidate[15] for drug delivery systems. In particular, MSNs can be functionalized with molecular, as well as supramolecular, switches in order to control the release of drug molecules in response to external stimuli. On-command release systems, which respond to a range of stimuli, including pH changes,[16] light initiation,[17] competitive binding,[18] redox activation,[19] biological triggers,[20] and temperature changes,[21] have been reported by us and others. To the best of our knowledge, however, all the on-command release systems reported to date cannot release multiple drugs in a step-by-step fashion.

Cyclodextrins (CDs), because of their abilities to form inclusion complexes with guest molecules,[22] have been the focus of much research. β-Cyclodextrin (β-CD), which comprises seven α-1,4-linked D-glucopyranosyl units with top and bottom cavities of 6.0 and 6.5 Å, respectively, has been employed as a gatekeeper in drug delivery systems.[23]

[*] Dr. C. Wang,[+] D. Cao, Dr. Y.-L. Zhao, J. W. Gaines, Dr. O. A. Bozdemir, M. W. Ambrogio, Dr. M. Frasconi, Dr. Y. Y. Botros, Prof. J. F. Stoddart
Center for the Chemistry of Integrated Systems
Department of Chemistry and Department of Material Sciences
Northwestern University
2145 Sheridan Road, Evanston, IL 60208 (USA)
E-mail: stoddart@northwestern.edu

Z. Li,[+] Prof. J. I. Zink
Department of Chemistry and Biochemistry and
California NanoSystems Institute, University of California
Los Angeles, 405 Hilgard Avenue, Los Angeles, CA 90095 (USA)
E-mail: zink@chem.ucla.edu

D. Cao, Prof. J. F. Stoddart
NanoCentury KAIST Institute and Graduate School of EEWS (WCU)
Korea Advanced Institute of Science and Technology (KAIST)
373-1 Guseong Dong, Yuseong Gu, Daejeon 305-701
(Republic of Korea)

Dr. Y. Y. Botros
Intel Labs, Building RNB-6-61
2200 Mission College Blvd., Santa Clara, CA 95054 (USA)
and
National Center for Nano Technology Research, King Abdulaziz City for Science and Technology (KACST)
P.O. BOX 6086, Riyadh 11442 (Kingdom of Saudi Arabia)

[+] These authors contributed equally to this work.

[**] The research at Northwestern University (NU) was enabled by the National Center for Nano Technology Research at the King Abdulaziz City for Science and Technology (KACST) in Saudi Arabia. The authors thank Dr. Turki S. Al-Saud and Dr. Mohamed B. Alfageeh at KACST for their generous support of this research. We also acknowledge support from the World Class University (WCU) Program (R-31-2008-000-10055-0) in Korea. The research at the University of California, Los Angeles (UCLA), was supported by the U.S. National Science Foundation under Grant CHE-0809384 and by the U.S. Department of Defense under Grant HDTRA1-08-1-0041. D.C. thanks the National Science Foundation for a Graduate Research Fellowship. We thank Aleksandr Bosoy for generating images for incorporation into the illustrations.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201107960.

The functionalization of MSN surfaces with β-CD rings provides us with the means to design a dual-cargo release system. The β-CD rings on the surface of the MSNs can serve as a gate for the storage of large (> 6.0 Å) molecules. Since the nanopores are not fully occupied by these large molecules, there is some space for small cargo molecules (< 6.0 Å) to diffuse into the nanopores through the cavities of the tethered β-CD rings. The small molecules are trapped inside the nanopores after plugging of the β-CD rings. The MSNs will, first of all, release the small molecules when the plugs are removed from the cavities of the β-CD rings, followed by release of the large molecules after cleavage of the β-CD rings from the surfaces of the MSNs.

Herein, we report an on-command dual-cargo release system (MSNs **1**) based on β-CD modified MSNs in which two cargos of differently sizes are loaded into MSNs **1** in sequence and then released in succession (Figure 1) by, first of all, lowering the pH and then by adding a reducing agent. The β-CD rings, persubstituted on their seven C6 positions and the seven linkers covalently connected to the surface by disulfide units, serve as the gatekeepers for large Hoechst 33342 molecules. The smaller *p*-coumaric acid (CA) is then diffused into the pore channels of the MSNs through the cavities of β-CD rings. The β-CD cavities can be plugged with methyl orange (MO), which was selected as the plugs because these molecules can be moved in and out of the cavities of the β-CD rings in response to changes in pH. The small molecules are released after protonation of MO, and the large ones are released on cleavage of the disulfide bonds.

Hoechst 33342 and CA were chosen as the fluorescent molecules to trace the independent release process because, firstly, the size of Hoechst 33342 (ca. 20 Å) and CA (ca. 4.5 Å) are optimal for them to act as the large and small molecules, respectively, and secondly, their fluorescent spectra are different. The synthesis (Figure 2) of MSNs **1** starts with the bare MCM-41 nanoparticles[24] which were, first of all, functionalized with 3-isocyanatopropyltriethoxysilane, before being reacted with propargyl ether **8** to produce the alkynyl-functionalized MSNs **2**. The FTIR spectrum (see the Supporting Information) of MSNs **2** shows a peak at 2150 cm^{-1}, which indicates the presence of C≡C bonds. The morphology of the MSNs **2** was confirmed (see the Supporting Information) by transmission electron microscopy (TEM) to consist of nanopores with average diameters of 2.5 nm. Following addition of per-6-azido-β-CD to the mixture of MSNs **2** and Hoechst 33342, the Hoechst 33342 loaded MSNs **3** were formed by a series of "click" reactions. CA was then allowed to diffuse through the cavities of the β-CD caps into the nanopores. After these procedures were complete, the β-CD openings were blocked with MO plugs.

Hoechst 33342 loaded MSNs **3**, in which the β-CD rings are linked covalently onto the MSN surfaces by using disulfide units, were synthesized. The mechanism of operation of the Hoechst 33342 loaded MSNs **3** is shown in Figure 3a. After cleavage of the disulfide bonds by a reducing agent, the Hoechst 33342 cargo will be released. This mechanism is invoked so that the release can be potentially triggered by glutathione from the cell cytosol,[25] such that the system would operate autonomously within living cells. It is worth

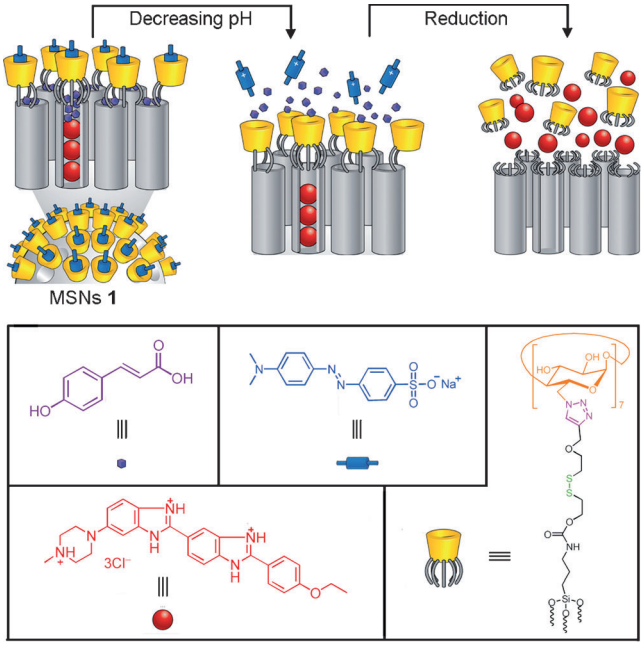

Figure 1. Schematic representation of the dual-cargo release process. The dual cargos can be released one step at a time by, first of all, lowering the pH and then adding mercaptoethanol. The β-CD rings are randomly distributed on the surfaces of MSNs **1**.

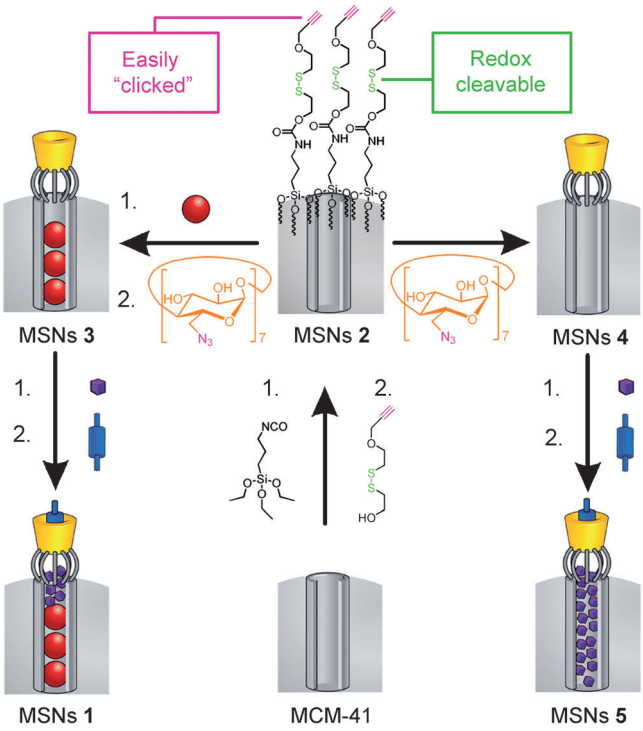

Figure 2. Synthetic procedures for preparing MSNs **1** and MSNs **5**. Utilizing supramolecular chemistry and mechanostereochemistry, dual cargos (Hoechst 33342 and CA) were loaded into MSNs **1** step-by-step. Although the β-CD rings are randomly distributed on the MSNs surfaces, the ideal mode is to have one nanopore functionalized with one β-CD ring according to the size match of the nanopores and the β-CD rings, as portrayed in the graphical representations.

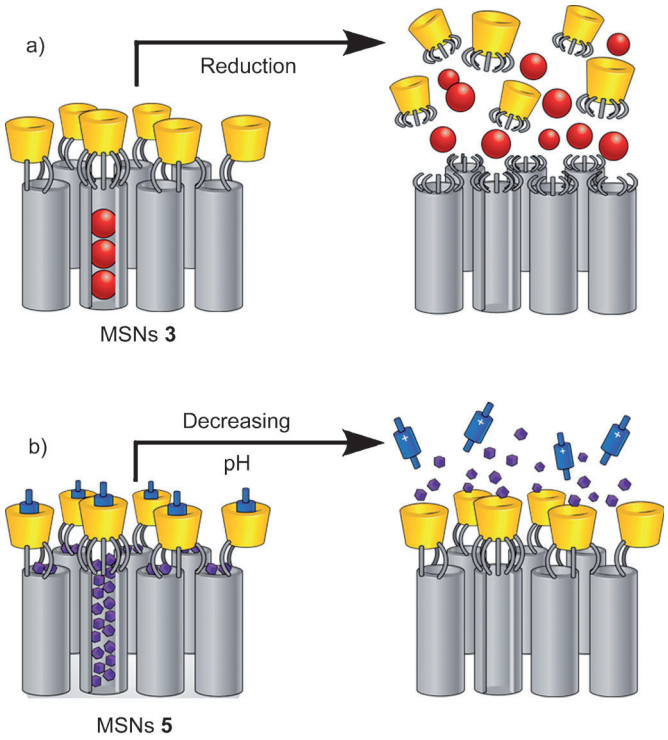

Figure 3. Schematic representation of the release process for a) MSNs **3**, which was prepared from per-6-azido-β-CD and alkynyl-functionalized MSNs **2** in the presence of Hoechst 33342 by means of a series of click reactions. b) MSNs **5**, which was prepared from β-CD-capped MSNs **4** and a MO plug in the presence of CA.

emphasizing that it is very important to wash the MSNs with acidic (pH 3) aqueous solution because the Hoechst 33342 dye can become adsorbed onto the outer surfaces of the MSNs only to dissociate from the MSN surface on lowering the pH. Hence, without an acidic wash, some Hoechst 33342 dye could be released on lowering the pH, while operating the dual-loaded system MSNs **1**, thus interfering with the independent release processes.

A sample of MSNs **3** was placed in the corner of a cuvette and water (pH 7) was slowly added into the cuvette without disturbing the MSNs. The release of Hoechst 33342 from the MSNs **3** was monitored by a 351 nm probe beam to track the fluorescence intensity of the released dye as a function of time. A flat baseline (Figure 4a) shows that the Hoechst 33342 molecules are held within the MSNs under neutral aqueous conditions, without any premature release. The pH of the solution was then lowered to 3.5 to confirm that a negligible amount of Hoechst 33342 is released simply by lowering the pH. Thereafter, 2-mercaptoethanol (ME) was used as the reductant to cleave the disulfide bonds. Upon addition of ME, the release of the Hoechst 33342 molecules was observed (Figure 4a) as a rapid increase of the fluorescence intensity around 500 nm as a function of time. These fluorescence intensity measurements were used to calculate that 0.20 μmol of Hoechst 33342 was released from 5 mg of MSNs **3** after approximately 2 h, corresponding to a release capacity of 2.5 wt%.

In order to test whether the CA cargo can diffuse into the MSNs through the cavities of the β-CD rings, we designed a pH-operated system (Figure 3b), namely MSNs **5**. On lowering the pH, the protonated MO molecules are expelled from the cavities of the β-CD ring and the CA molecules are released. There are three reasons for choosing MO as the plug to form a complex with β-CD. Firstly, the formation constant (K_f) for the complex[26] between β-CD and MO[27] is 4550 M^{-1} at pH 7, that is, it is much stronger than that (408 M^{-1}, see the Supporting Information) of β-CD with CA. Secondly, the K_f of β-CD with MO decreases[27] to 292 M^{-1} at pH 2, resulting in the release of cargo. These pH-responsive MSNs may potentially be interfaced with biological systems, because the lysosomal pH levels in cancer cells are somewhat lower[14c] than the pH levels in healthy cells. Finally, the existence of the protonated MO does not interfere with the tracing of either Hoechst 33342 or CA.

The synthetic protocol for the production of MSNs **5** is summarized in Figure 2. After reaction between MSNs **2** with its propargyl ether and per-6-azido-β-CD in a series of click reactions, the β-CD-capped MSNs **4** were isolated and characterized by TEM and ^{13}C and ^{29}Si cross-polarization magic-angle-spinning (CP-MAS) solid-state NMR spectroscopy. The TEM (see the Supporting Information) results show that the shapes of the MSNs **4** experience no obvious changes compared to those of bare MCM-41. The ^{13}C and ^{29}Si CP-MAS solid-state NMR spectra (see the Supporting Information) demonstrate the successful functionalization of MSNs with β-CD rings. The MSNs **5** were finally obtained after the MSNs **4** were loaded with CA molecules and capped by MO plugs.

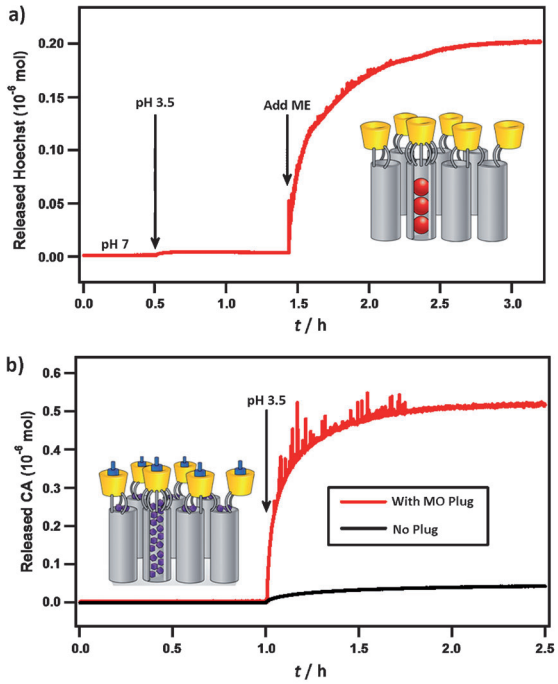

Figure 4. Release profiles of a) MSNs **3** while monitoring emission of Hoechst 33342 at 500 nm following the addition of ME. b) MSNs **5** and CA-loaded nanoparticles without the MO plugs, while monitoring emission CA after lowering the pH at 425 nm.

The operation of the MSNs **5** was followed (Figure 4b) by fluorescence spectroscopy. A flat baseline at pH 7 indicates that no CA is released under neutral conditions. When the pH of the solution was adjusted[28] to 3.5 by the addition of HCl solution (0.1M), a rapid increase in the emission intensity around 425 nm occurred showing that the CA is released from the nanopores of the MSNs upon lowering the pH. UV/Vis spectrophotometery indicated that around 0.52 μmol of the CA was released from 5 mg of MSNs **5**, corresponding to a release capacity of approximately 1.7 wt%. In a control experiment, CA-loaded nanoparticles without the MO plugs also registered a release (see Figure 4b and the Supporting Information) of CA on lowering the pH to 3.5. Since CA shows affinity for β-CD, it can also be used as a plug. The release capacity, however, corresponds to a maximum of approximately 0.14%, which is much lower than that of the CA-loaded MSNs **5**. The reason for this difference lies in the fact that the CA has a much lower K_f compared to that of MO with β-CD, therefore CA can escape more readily through the β-CD rings during the washing process than observed for MSNs **5**. We also prepared CA-loaded MSNs capped by a 1-adamantylamine plug. In this case, no changes in fluorescence (see the Supporting Information) were observed, even after lowering the pH to 3. This observation can be explained by the strong K_f[22b] (ca. 10^4) between protonated 1-adamantylamine and β-CD, and reflects a situation in which even protonated 1-adamantylamine will remain in the β-CD, thus preventing the small molecules from being released.

Thus, it is clear that changing the pH can induce the release of CA, whereas the release of Hoechst 33342 can only be triggered by the addition of ME. We synthesized the MSNs **1** (Figure 2) by loading Hoechst 33342 and CA step-by-step. The dual release of the molecules from the dye-loaded MSNs **1** was monitored by fluorescence spectroscopy. The CA molecules were released first on lowering the pH of the solution, an observation that was confirmed (Figure 5a) by the release profile. The fluorescence spectra (Figure 5b) for the solution before and after CA release were dramatically different. According to the UV/Vis absorption measurements (see the Supporting Information), approximately 0.13 μmol of the CA is released, corresponding to a 0.42 wt% release capacity. After this first release process, since the existence of the released CA in solution may interfere with the monitoring of the subsequent release process upon ME addition, we removed the solution above the MSNs and carefully added fresh water into the cuvette. Again, a flat baseline shows that negligible Hoechst 33342 is released from the MSNs into pH 7 aqueous media. The release profile (Figure 5a) indicates that the Hoechst 33342 molecules can only be released upon ME addition, with the calculated released amount being approximately 0.22 μmol. The fluorescence spectra are shown (Figure 5c) for the solution before and after the Hoechst 33342 release. These results demonstrate very clearly that MSNs **1** can hold two cargos successfully and, more importantly, release CA first, after reducing the pH, and then release Hoechst 33342, after addition of ME.

In summary, we have designed and synthesized a dual-cargo release system in which two differently sized molecular cargos can be loaded into MSNs and then subsequently released in sequence when triggered by two different stimuli. The β-CD rings on the MSN surface not only act as gatekeepers for the larger molecules but they also allow small molecules to diffuse into the inside of the nanopore channels only to find themselves capped by plugs. Fluorescent molecules were chosen as the molecular cargos in order to establish a proof-of-principle operation for this integrated functional nanosystem. The results demonstrate that the smaller molecules are released first of all by lowering the pH, and then the larger ones follow upon cleavage of the disulfide bonds.

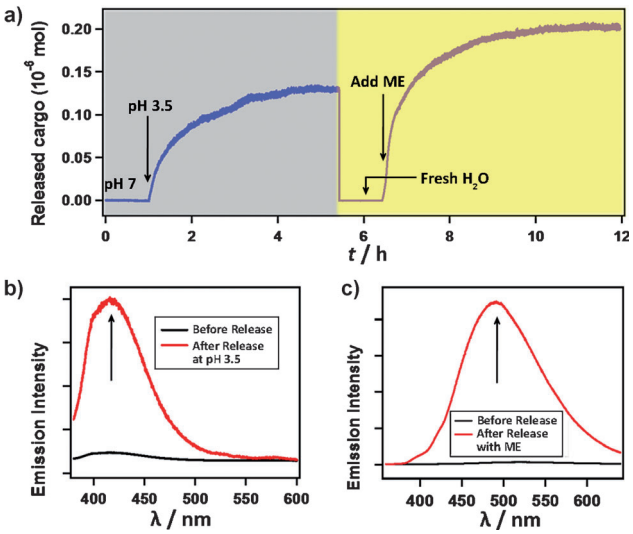

Figure 5. a) Step-by-step release profile of dual-cargo-loaded MSNs **1** by lowering the pH (monitored at 425 nm) and then adding ME (monitored at 500 nm). Fluorescence spectra of dual-cargo-loaded MSNs **1** b) before (black) and after (red) lowering the pH to 3.5, c) followed by changing the solution to fresh water (black) and addition of ME (red).

This dual-drug delivery system has the potential to treat human diseases where combination therapies are desirable. In view of the lower lysosome pH levels and the presence of higher concentrations of glutathione in cancer cells, further investigations will employ this integrated functional nanosystem to deliver two anticancer drugs (i.e., cisplatin and doxorubicin) of different sizes before performing in vivo dual-drug release experiments.

Experimental Section

MSNs 3. MSNs **2** (50 mg; see the Supporting Information) were soaked in an aqueous solution of Hoechst 333422 (1 mL, 3.0 mM) overnight at RT. A solution of per-6-azido-β-cyclodextrin (0.03 g, 0.3 mmol) in DMF (2 mL) was added, followed by sodium ascorbate (2 mg, 0.01 mmol) and $CuSO_4 \cdot 5H_2O$ (0.75 mg, 3 μmol). The reaction mixture was left to stir for 3 days at RT after which the nanoparticles were filtered and washed with copious amounts of MeOH, acidic H_2O (pH 3), and H_2O, before being dried under vacuum to afford MSNs **3**, which were used immediately for laser investigations involving fluorescence spectroscopy and release profiles.

MSNs 5. β-CD-Capped MSNs **4** (50 mg; see the Supporting Information) were added to an aqueous solution of CA (1 mL, 1.0 mM). The resulting suspension was shaken at RT for 24 h to allow

the CA to diffuse into the nanopores of the nanoparticles which were centrifuged to remove the loading solution. MO (50 mg) was added to the pellet that was resuspended in H_2O (0.5 mL). After the suspension had been left on a shaker for an additional 24 h, the MSNs **5** were obtained by centrifugation, washed with H_2O (10 times), and dried under vacuum. Release studies using them were carried out immediately.

MSNs 1. MSNs **3** (50 mg) were soaked in an aqueous solution of CA (1 mL, 1.0 mM) at RT for 24 h. The loaded nanoparticles were then centrifuged to remove the loading solution. MO (50 mg, 0.15 mmol) was added to the pellet that was resuspended in H_2O (0.5 mL). After the mixture had been left on a shaker for a further 24 h, the resulting MSNs **1** were collected by centrifugation, washed with H_2O (10 times), and dried under vacuum. Release studies using them were carried out immediately.

Release Studies. Cargo-loaded nanoparticles were examined by using the spectroscopic setup illustrated in the Supporting Information. A sample of nanoparticles (5 mg) was placed in the corner of an 8 mL quartz cuvette, and a 1 mm stirring bar was placed at the bottom of the cuvette. Water (pH 7, 2 mL) was added in a dropwise fashion in order to prevent the nanoparticles from dispersing into the solution. The solution in the cuvette was stirred slowly after the addition of water. An excitation beam of 351 nm was directed at the solution above the nanoparticles, and the fluorescence intensity of the released molecules was collected at 1 s intervals during the course of the experiment. Activation of cargo release form the nanoparticles was accomplished by 1) adjusting the pH of the solution by addition of either HCl solution (1.0 M) or mercaptoethanol (1 mL), or by 2) adjusting the pH, followed by addition of mercaptoethanol.

Received: November 11, 2011
Revised: March 7, 2012
Published online: April 13, 2012

Keywords: β-cyclodextrins · cargo release · drug delivery · nanostructures · nanotechnology

[1] J. Jia, F. Zhu, X. Ma, Z. W. Cao, Y. X. Li, Y. Z. Chen, *Nature* **2009**, *458*, 111 – 128.
[2] K. d. G. Donati, R. Rabagliati, L. Iacoviello, R. Cauda, *Lancet Infect. Dis.* **2004**, *4*, 213 – 222.
[3] W. L. Suarez-Pinzon, R. F. Power, Y. Yan, C. Wasserfall, M. Atkinson, A. Rabinovitch, *Diabetes* **2008**, *57*, 3281 – 3288.
[4] D. Lane, *Nat. Biotechnol.* **2006**, *24*, 163 – 164.
[5] a) R. Langer, *Science* **1990**, *249*, 1527 – 1533; b) T. M. Allen, P. R. Cullis, *Science* **2004**, *303*, 1818 – 1822; c) J. Shi, A. R. Votruba, O. C. Farokhzad, R. Langer, *Nano Lett.* **2010**, *10*, 3223 – 3230.
[6] K. E. Uhrich, S. M. Cannizzaro, R. S. Langer, K. M. Shakesheff, *Chem. Rev.* **1999**, *99*, 3181 – 3198.
[7] C. C. Lee, J. A. MacKay, J. M. J. Fréchet, F. C. Szoka, *Nat. Biotechnol.* **2005**, *23*, 1517 – 1526.
[8] K. Kataoka, A. Harada, Y. Nagasaki, *Adv. Drug Delivery Rev.* **2001**, *47*, 113 – 131.
[9] J. J. Moon, H. Suh, A. Bershteyn, M. T. Stephan, H. Liu, B. Huang, M. Sohail, S. Luo, S. H. Um, H. Khant, J. T. Goodwin, J. Ramos, W. Chiu, D. J. Irvine, *Nat. Mater.* **2011**, *10*, 243 – 251.
[10] I. Brigger, C. Dubernet, P. Couvreur, *Adv. Drug Delivery Rev.* **2002**, *54*, 631 – 651.
[11] a) Y. Zhao, B. G. Trewyn, I. I. Slowing, V. S. Y. Lin, *J. Am. Chem. Soc.* **2009**, *131*, 8398 – 8400; b) C. E. Ashley, E. C. Carnes, G. K. Phillips, D. Padilla, P. N. Durfee, P. A. Brown, T. N. Hanna, J. Liu, B. Phillips, M. B. Carter, N. J. Carroll, X. Jiang, D. R. Dunphy, C. L. Willman, D. N. Petsev, D. G. Evans, A. N. Parikh, B. Chackerian, W. Wharton, D. S. Peabody, C. J. Brinker, *Nat. Mater.* **2011**, *10*, 389 – 397.
[12] a) N. Kolishetti, S. Dhar, P. M. Valencia, L. Q. Lin, R. Karnik, S. J. Lippard, R. Langer, O. C. Farokhzad, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 17939 – 17944; b) T. Okuda, K. Tominaga, S. Kidoaki, *J. Controlled Release* **2010**, *143*, 258 – 264.
[13] a) A. Stein, B. J. Melde, R. C. Schroden, *Adv. Mater.* **2000**, *12*, 1403 – 1419; b) S. Angelos, E. Johansson, J. F. Stoddart, J. I. Zink, *Adv. Funct. Mater.* **2007**, *17*, 2261 – 2271; c) M. Liong, S. Angelos, E. Choi, K. Patel, J. F. Stoddart, J. I. Zink, *J. Mater. Chem.* **2009**, *19*, 6251 – 6257; d) K. K. Cotí, M. E. Belowich, M. Liong, M. W. Ambrogio, Y. A. Lau, H. A. Khatib, J. I. Zink, N. M. Khashab, J. F. Stoddart, *Nanoscale* **2009**, *1*, 16 – 39; e) J. L. Vivero-Escoto, I. I. Slowing, B. G. Trewyn, V. S-Y. Lin, *Small* **2010**, *6*, 1952 – 1967; f) M. Manzano, M. Vallet-Regi, *J. Mater. Chem.* **2010**, *20*, 5593 – 5604; g) S. H. Wu, Y. Hung, C. Y. Mou, *Chem. Commun.* **2011**, *47*, 9972 – 9985.
[14] a) M. Liong, J. Lu, M. Kovochich, T. Xia, S. G. Ruehm, A. E. Nel, F. Tamanoi, J. I. Zink, *ACS Nano* **2008**, *2*, 889 – 896; b) J. Lu, E. Choi, F. Tamanoi, J. I. Zink, *Small* **2008**, *4*, 421 – 426; c) J. M. Rosenholm, A. Meinander, E. Peuhu, R. Niemi, J. E. Eriksson, C. Sahlgren, M. Linden, *ACS Nano* **2009**, *3*, 197 – 206; d) H. Meng, M. Xue, T. Xia, Y.-L. Zhao, F. Tamanoi, J. F. Stoddart, J. I. Zink, A. E. Nel, *J. Am. Chem. Soc.* **2010**, *132*, 12690 – 12697.
[15] a) M. Vallet-Regí, F. Balas, D. Arcos, *Angew. Chem.* **2007**, *119*, 7692 – 7703; *Angew. Chem. Int. Ed.* **2007**, *46*, 7548 – 7558; b) I. I. Slowing, J. L. Vivero-Escoto, C. W. Wu, V. S. Y. Lin, *Adv. Drug Delivery Rev.* **2008**, *60*, 1278 – 1288; c) M. W. Ambrogio, C. R. Thomas, Y. L. Zhao, J. I. Zink, J. F. Stoddart, *Acc. Chem. Res.* **2011**, *44*, 903 – 913; d) J. E. Lee, N. Lee, T. Kim, J. Kim, T. Hyeon, *Acc. Chem. Res.* **2011**, *44*, 893 – 902; e) Z. Li, J. C. Barnes, A. Bosoy, J. F. Stoddart, J. I. Zink, *Chem. Soc. Rev.* **2012**, *41*, 2590 – 2605.
[16] a) C. Park, K. Oh, S. C. Lee, C. Kim, *Angew. Chem.* **2007**, *119*, 1477 – 1479; *Angew. Chem. Int. Ed.* **2007**, *46*, 1455 – 1457; b) S. Angelos, Y. W. Yang, K. Patel, J. F. Stoddart, J. I. Zink, *Angew. Chem.* **2008**, *120*, 2254 – 2258; *Angew. Chem. Int. Ed.* **2008**, *47*, 2222 – 2226; c) L. Du, S. Liao, H. A. Khatib, J. F. Stoddart, J. I. Zink, *J. Am. Chem. Soc.* **2009**, *131*, 15136 – 15142; d) H. P. Rim, K. H. Min, H. J. Lee, S. Y. Jeong, S. C. Lee, *Angew. Chem.* **2011**, *123*, 9015 – 9019; *Angew. Chem. Int. Ed.* **2011**, *50*, 8853 – 8857.
[17] a) Y. Zhu, M. Fujiwara, *Angew. Chem.* **2007**, *119*, 2291 – 2294; *Angew. Chem. Int. Ed.* **2007**, *46*, 2241 – 2244; b) S. Angelos, E. Choi, F. Vögtle, L. De Cola, J. I. Zink, *J. Phys. Chem. C* **2007**, *111*, 6589 – 6592; c) D. P. Ferris, Y. L. Zhao, N. M. Khashab, H. A. Khatib, J. F. Stoddart, J. I. Zink, *J. Am. Chem. Soc.* **2009**, *131*, 1686 – 1688; d) S. Angelos, Y. W. Yang, N. M. Khashab, J. F. Stoddart, J. I. Zink, *J. Am. Chem. Soc.* **2009**, *131*, 11344 – 11346; e) J. L. Vivero-Escoto, I. I. Slowing, C.-W. Wu, V. S. Y. Lin, *J. Am. Chem. Soc.* **2009**, *131*, 3462 – 3463.
[18] K. C. F. Leung, T. D. Nguyen, J. F. Stoddart, J. I. Zink, *Chem. Mater.* **2006**, *18*, 5919 – 5928.
[19] a) C.-Y. Lai, B. G. Trewyn, D. M. Jeftinija, K. Jeftinija, S. Xu, S. Jeftinija, V. S. Y. Lin, *J. Am. Chem. Soc.* **2003**, *125*, 4451 – 4459; b) T. D. Nguyen, H.-R. Tseng, P. C. Celestre, A. H. Flood, Y. Liu, J. F. Stoddart, J. I. Zink, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 10029 – 10034; c) K. Patel, S. Angelos, W. R. Dichtel, A. Coskun, Y.-W. Yang, J. I. Zink, J. F. Stoddart, *J. Am. Chem. Soc.* **2008**, *130*, 2382 – 2383; d) M. W. Ambrogio, T. A. Pecorelli, K. Patel, N. M. Khashab, A. Trabolsi, H. A. Khatib, Y. Y. Botros, J. I. Zink, J. F. Stoddart, *Org. Lett.* **2010**, *12*, 3304 – 3307.
[20] a) C.-L. Zhu, C.-H. Lu, X.-Y. Song, H.-H. Yang, X.-R. Wang, *J. Am. Chem. Soc.* **2011**, *133*, 1278 – 1281; b) N. Singh, A. Karambelkar, L. Gu, K. Lin, J. S. Miller, C. S. Chen, M. J. Sailor, S. N. Bhatia, *J. Am. Chem. Soc.* **2011**, *133*, 19582 – 19585.
[21] a) A. Schlossbauer, S. Warncke, P. M. E. Gramlich, J. Kecht, A. Manetto, T. Carell, T. Bein, *Angew. Chem.* **2010**, *122*, 4842 – 4845; *Angew. Chem. Int. Ed.* **2010**, *49*, 4734 – 4737; b) E. Aznar, L. Mondragón, J. V. Ros-Lis, F. Sancenón, M. D. Marcos, R.

Martínez-Máñez, J. Soto, E. Pérez-Payá, P. Amorós, *Angew. Chem.* **2011**, *123*, 11368 – 11371; *Angew. Chem. Int. Ed.* **2011**, *50*, 11172 – 11175.

[22] a) F. Cramer, *Einschlussverbindungen*, Springer, Berlin, **1954**; b) D. French, *Adv. Carbohydr. Chem.* **1957**, *12*, 189 – 260; c) F. Cramer, *Angew. Chem.* **1956**, *68*, 115 – 120; d) M. L. Bender, M. Komiyama, *Cyclodextrin Chemistry*, Springer, Berlin, **1978**; e) R. Breslow, *Chem. Soc. Rev.* **1972**, *1*, 553 – 580; f) J. Szejtli, *Cyclodextrin Technology*, Kluwer Academic Publishers, Dordrecht, **1988**; g) W. Saenger, *Angew. Chem.* **1980**, *92*, 343 – 361; *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 344 – 362; h) I. Tabushi, *Pure Appl. Chem.* **1986**, *58*, 1529 – 1534; i) J. F. Stoddart, *Carbohydr. Res.* **1989**, *192*, xii – xv; j) K. A. Connors, *Chem. Rev.* **1997**, *97*, 1325 – 1358; k) M. V. Rekharsky, Y. Inoue, *Chem. Rev.* **1998**, *98*, 1875 – 1918; l) H. Dodziuk, *Cyclodextrins and Their Complexes*, Wiley-VCH, Weinheim, **2006**; m) Y. Chen, Y. Liu, *Chem. Soc. Rev.* **2010**, *39*, 495 – 505; n) R. Breslow in *Molecular Encapsulation* (Eds.: U. Berinker, J.-L. Mieusset), Wiley, Chichester, **2010**, pp. 43 – 65.

[23] a) H. Kim, S. Kim, C. Park, H. Lee, H. J. Park, C. Kim, *Adv. Mater.* **2010**, *22*, 4280 – 4283; b) C. Park, K. Lee, C. Kim, *Angew. Chem.* **2009**, *121*, 1301 – 1304; *Angew. Chem. Int. Ed.* **2009**, *48*, 1275 – 1278; c) Y.-L. Zhao, Z. Li, S. Kagehie, Y. Y. Botro, J. F. Stoddart, J. I. Zink, *J. Am. Chem. Soc.* **2010**, *132*, 13016 – 13025.

[24] The MCM-41 mesoporous silica nanoparticles were synthesized using a surfactant-templated sol–gel process (see Ref. [14a]). The templating surfactant was removed by acid extraction, and the morphology of the resulting particles was characterized by transmission electron microscopy TEM and X-ray diffraction (XRD; see the Supporting Information). The spherical particles are around 100 nm in diameter and display hexagonal arrays of pore channels of around 2.5 nm.

[25] a) R. Hong, G. Han, J. M. Fernández, B. Kim, N. S. Forbes, V. M. Rotello, *J. Am. Chem. Soc.* **2006**, *128*, 1078 – 1079; b) S. Takae, K. Miyata, M. Oba, T. Ishii, N. Nishiyama, K. Itaka, Y. Yamasaki, H. Koyama, K. Kataoka, *J. Am. Chem. Soc.* **2008**, *130*, 6001 – 6009.

[26] Although the binding constant was obtained in solution state, we assume that the sites of host – guest binding between the plugs and the β-CD rings are equivalent on surfaces. See: M. J. W. Ludden, X. Li, J. Greve, A. Amerongen, M. Escalante, V. Subramaniam, D. N. Reinhoudt, J. Huskens, *J. Am. Chem. Soc.* **2008**, *130*, 6964 – 6973.

[27] Y. Liu, C.-C. You, S. He, G.-S. Chen, Y.-L. Zhao, *J. Chem. Soc. Perkin Trans. 2* **2002**, 463 – 469.

[28] The pK_a of MO is 3.47. MO has been used as pH indicator and it shows a color transition between pH 3.1–4.4.

