Drug Delivery

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Polyvalent Nucleic Acid/Mesoporous Silica Nanoparticle Conjugates: Dual Stimuli-Responsive Vehicles for Intracellular Drug Delivery**

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The distinctive characteristics of mesoporous silica nanoparticles (MSPs) such as thermal stability, tunable pore sizes, large load capacity, and the ease of surface functionalization make these scaffolds ideal for the design of nanodevices and "on-command" delivery applications.[1] To date, several MSP-based controlled-release systems have been synthesized by using different kinds of capping agents including organic molecules,[2] nanoparticles,[3] and supramolecular assemblies.[4] "On-demand" release systems that respond to a range of stimuli, including redox,[2b,4a,5] pH or temperature,[6] enzymes,[7] competitive binding,[8] and photoirradiation[2a,4b,9] have recently been reported. Despite these burgeoning achievements, many of the existing capping systems have disadvantages such as the use of stimuli that are complicated and/or difficult to apply, poor applicability in aqueous solutions and biocompatibility, and the toxicity of the capping agents used. In particular, regardless of recent reports on capped MSPs that can be uncapped by certain enzymes[7] or carbohydrates,[10] the utility of MSP-based devices involving biomolecules for real delivery systems is still in its infancy. Therefore, the search for effective systems that, in particular, respond to internal biological stimuli still remains a big challenge in this field.

Herein we describe the design and construction of a stimuli-responsive vehicle for intracellular drug delivery using a polyvalent nucleic acid/MSP "click" conjugate that responds to both external and endogenous activation. Nucleic acids have been recognized as attractive building blocks for nanotechnology and materials science owing to the remarkable specificity and versatility of these units.[11] The unique structural motif and self-recognition properties of duplex DNA, including temperature-dependent assembly, as well as the enzymatic recognition of specific encoded bases, may be applied as triggers for functional DNA manipulation. As shown in Figure 1, self-complementary duplex DNA was anchored to the openings of the MSPs and was utilized as a cap for trapping the guest molecules within the porous channels. The duplex DNA cap could be either denatured by heating or hydrolyzed by endonucleases, thus opening the nanopores and releasing the cargo. As a proof-of-principle experiment, rhodamine B was chosen as model molecule and deoxyribonuclease I (DNase I) was utilized as a representative endonuclease for DNA degradation. The opening of the capped system was tested by measuring the stimuli-triggered dye release from the MSPs. Importantly, we have demonstrated the successful loading of anticancer drugs camptothecin (CPT) and floxuridine (FUDR) into MSPs, and efficient intracellular controlled drug delivery in human cancer cells when endogenous nuclease was used as a stimulus.

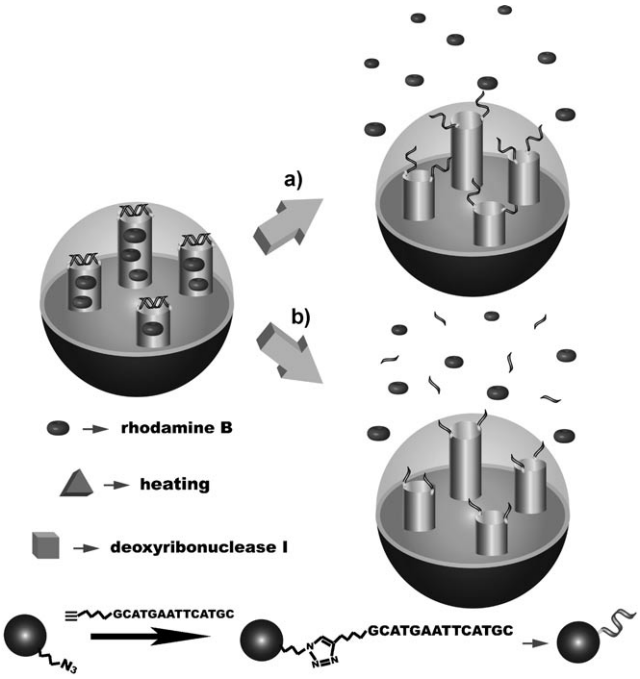

Figure 1. Release of guest molecules from the pores of DNA-capped MSPs upon a) heating and b) treatment with DNase I.

MCM-41 silica nanoparticles were prepared by following a base-catalyzed sol–gel procedure,[12] and the resulting porous silica nanoparticles (100 nm in diameter) that contain hexagonally arranged pores were characterized by SEM and X-ray diffraction (Figure S1 and S2 in the Supporting Information). The N_2 adsorption–desorption isotherms of

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MCM-41 particles showed a typical type IV curve with a specific surface area of 1006 $m^2 g^{-1}$, an average pore diameter of 3.0 nm, and a narrow pore distribution (Figure S3 and Table S1 in the Supporting Information). The copper(I)-catalyzed 1,3-dipolar cycloaddition reaction has found applications in a wide variety of areas, including materials chemistry and pharmaceutical sciences. This reaction provides a facile and versatile method to join different molecules together in high yields.[13] Indeed, this method has been applied to conjugate various compounds onto the surface of MSPs.[6c,7a,14] We then sought to take advantage of this unique feature to covalently modify the mesoporous silica surface with intact DNA. The surface of the MSPs was first treated with 3-chloropropyltrimethoxysilane (CPTMS) to afford MSPs-Cl. The resulting silica particles were functionalized with sodium azide in *N*,*N*-dimethylformamide (DMF),[15] and the resulting azide-functionalized particles (denoted as MSPs-N_3) were then reacted with pre-hybridized hexynyl-modified DNA in a copper(I)-catalyzed cycloaddition reaction at 4 °C to produce MSNs-DNA. The surface functionalization of MSPs was monitored by FTIR spectroscopy (Figure 2). The

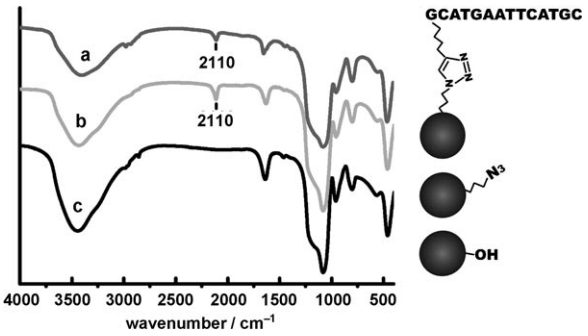

Figure 2. FTIR spectra of a) MCM-41, b) MSPs-N_3, and c) MSPs-DNA.

successful grafting of DNA onto the mesoporous silica was validated by the apparent decline of absorption band around 2110 cm^{-1}, which is assigned to the azide stretch. Quantification of the density of DNA anchored on MSNs-DNA was accomplished by thermogravimetric analysis (TGA) and element analysis (Figure S4 and Table S2 in the Supporting Information), which corresponded to an immobilization efficiency of approximately 10.54 μmol g^{-1} SiO_2.

To investigate the stimuli-triggered controlled release of the MSPs-DNA system, dye loading was accomplished by soaking MSPs-N_3 in a solution of rhodamine B to allow the guest molecules to diffuse into the pores of the MSPs. The closure reaction was performed by the addition of pre-hybridized hexynyl-modified DNA using the copper(I)-catalyzed click reaction to block the pores (Figure S5 in the Supporting Information). The excess dye was removed by centrifugation and repeated washing with tris(hydroxymethyl)-aminomethane buffer (Tris-HCl; pH 7.4). A small sample (3 mg) of the resulting particles (denoted as MSPs-RhB) was placed in a cuvette, which was then carefully filled with Tris-HCl buffer (6 mL, 20 mM Tris-HCl, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.4). The absorbance maximum of

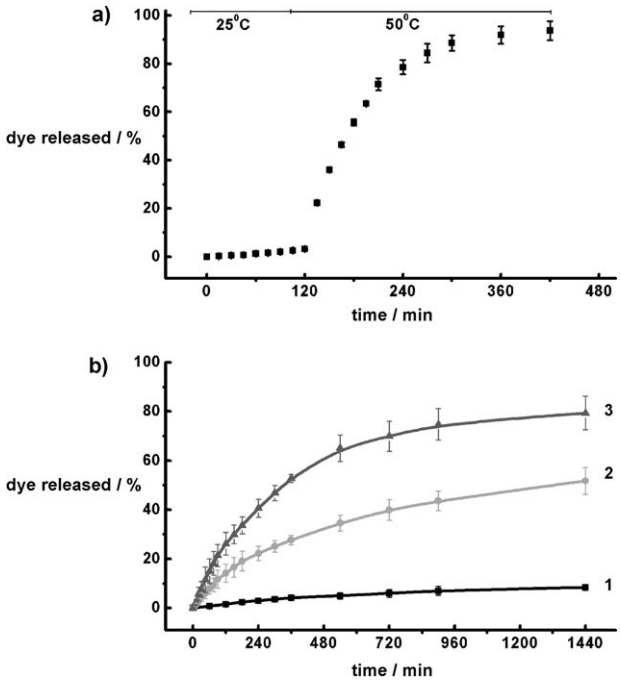

Figure 3. Release profiles of rhodamine B from MSPs-DNA a) triggered by thermal denaturation, and b) triggered by enzymatic hydrolysis 1) without external stimuli, and in the presence of 2) 10 U mL^{-1} DNase I, or 3) 20 U mL^{-1} DNase I.

rhodamine B (553 nm) was plotted as a function of time to generate a release profile. As shown in Figure 3a, the absorbance of rhodamine was essentially constant in the absence of external stimuli at 25 °C, thus indicating that duplex DNA acted as an efficient cap for retention of guest molecules with negligible leakage. Since the diameter of the B-form duplex DNA structure is 2.0 nm, the interconnected structure may be inaccessible for small molecules such as rhodamine B, which have approximate sizes of 1.5 nm, as the size of the pore aperture is clearly reduced. On the other hand, an increase of the temperature to 50 °C resulted in opening of the attached duplex caps by thermal denaturation, and led to the fast release of the loaded rhodamine molecules (Figure 3a and Figure S6 in the Supporting Information). The temperature-dependent release rate is consistent with the mechanism of operation of the MSP system: the release of guest molecules depends on the conformational change from duplex to random-coil DNA. Furthermore, the thermoresponsive release was observed immediately after stimulation because of the high degree of molecular cooperation between individual DNA molecules. Our results confirmed that the rigidity of short segments of the double helix and the flexibility of single-stranded segments make DNA an ideal material for construction of thermally responsive release devices. Although the temperature required (50 °C) in the present system is not appropriate for physiological temperatures, this proof of concept might provide a principle for the design of nanodevices that use the distinctive sequence-specific properties of DNA to respond to variations in physiological temperature. The addition of DNase I also activates the capped system (Figure 3b). The amount of dye

released reached about 81% after 24 hours incubation upon the introduction of 20 U mL^{-1} DNase I, whereas only 55% release was obtained in the same amount of time with 10 U mL^{-1} DNase I. Meanwhile, it is apparent that the dye release increased as the DNase I concentration increased, thus demonstrating another advantage as the capped system could discriminate the activity of DNase I. Release of the encapsulated dyes depends on the enzymatic degradation of duplex DNA interconnects as a consequence of cleavage of the phosphodiester bonds. It was also found that the release of rhodamine was negligible at acidic pH values (20 mM 4-morpholineethanesulfonic acid (MES), 100 mM NaCl, pH 5.0), thus suggesting that the nanoparticles are stabile under simulated cancer cell conditions (Figure S7 in the Supporting Information). In addition, unfunctionalized MCM-41 and MSPs functionalized with random-sequence DNA showed sustained dye release in Tris buffer (pH 7.4), thus further supporting the conclusion that the duplex DNA structure has a direct effect on the accessibility of the pores (Figures S7 and S8 in the Supporting Information).

For many practical drug delivery systems, "zero-premature release" and "stimuli-responsive controlled release" of the pharmaceutical cargo are two very important prerequisites that impact the therapeutic efficacy and cytotoxicity of drug delivery. To verify the feasibility of the MSPs-DNA system for intracellular therapeutic applications, camptothecin (CPT), which is a hydrophobic anticancer drug, was chosen as guest molecule for a controlled release study in human liver cancer cells (HepG2). Endonucleases, which are a family of nucleases that can hydrolyze the internal phosphodiester bonds in DNA or RNA, are ubiquitous in most organisms and are of vital importance in molecular biology.[16] Therefore, the presence of endonucleases in the cells should favor the cleavage of the DNA attached to outer shells to allow the release of the drug molecules. To visualize intracellular delivery of CPT, fluorescein isothiocyanate (FITC) was incorporated covalently into the silica walls by following a reported procedure in order to provide an internal standard.[17] Suspensions of CPT-loaded FITC-labeled MSPs-DNA, CPT-loaded FITC-labeled MSPs, FITC-labeled MSPs, or CPT in phosphate-buffered saline (PBS) were introduced into the culture medium overnight to mimic the blood circulation process prior to the cellular uptake. As CPT emits strong blue luminescence under excitation with UV light,[18] and the excitation wavelength is different from that of the fluorescein, fluorescence microscopy was used to monitor the endocytosis of CPT in cancer cells (Figure 4). Bright green fluorescence was observed for cells treated with CPT-loaded MSPs-DNA, CPT-loaded MSPs, and nonloaded MSPs when excited at 435 nm after 3 hours of incubation, thus suggesting that each material could be taken up rapidly by the cancer cells. By contrast, the cells that treated with CPT-loaded MSPs-DNA showed significant blue luminescence under UV excitation, while only faint blue fluorescence was observed when CPT-loaded MSPs were used. The results revealed that CPT-loaded MSPs-DNA allowed more efficient drug delivery with minimal leakage into the culture medium compared to the CPT-loaded MSPs. The fluorescence ratio ($I_{423\,nm}/I_{519\,nm}$) of HepG2 cells with each material correlates with their endocytosis efficiency (Figure 4i), and the intracellular concentration of CPT might also be quantified with a standard curve (Figure S9 in the Supporting Information). The facile internalization of the negatively charged MSPs-DNA might be the result of several factors, including the nanoparticle concentration, DNA density, and protein absorption during the cell culture.[19] In addition, the cells remained nonfluorescent in the presence of free CPT suspended in PBS, thus indicating that the drug was not internalized by the cells because of its poor solubility. Delivery of CPT into the cancer cells led to growth inhibition and cell death (Figure 4j). Cell viability was analyzed by the MTT assay (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), which revealed that CPT suspended in PBS was nontoxic to cells, even at high concentrations. However, growth inhibition of cancer cells was observed when the cells were treated with CPT-loaded nanoparticles. In contrast to CPT-loaded MSPs, the remarkably higher cytotoxic efficacy of the CPT-loaded MSPs-DNA further confirmed that the DNA-capped nanoparticles were capable of delivering drug molecules into cancer cells in response to endonucleases. The process occurred with negligible leakage, which led to a remarkably enhanced efficiency in killing cancer cells. Finally, we also demonstrated the applicability of the MSP vehicle for intracellular delivery of soluble drug floxiuridine (FUDR; Figure S10 in the Supporting Information).

In summary, we have designed and synthesized a drug delivery vehicle that is based on DNA-MSPs conjugates and responds to different stimuli. A self-complementary duplex DNA was attached directly to the outlet of the mesopores by the highly efficient copper(I)-catalyzed azide–alkyne reaction and served as a cap to entrap guest molecules within the mesopores. Cargo release was triggered either by thermal denaturation of the DNA duplex or by the introduction of DNase I to cleave the DNA with high selectivity. Moreover, we have successfully demonstrated that DNA-capped nanoparticles showed a remarkably enhanced efficiency in killing cancer cells, as drug molecules were delivered upon stimulus by endonucleases. The good biocompatibility, cellular uptake properties, and efficient intracellular drug release provide a basis for in vivo controlled-release biomedical applications. This proof of concept might pave the way for a new generation of carrier materials and could also provide a general route for the use of other functional nucleic acids as capping agents in the field of versatile controlled delivery nanodevices. In principle, the distinctive sequence-specific properties of DNA could enable the design of nanocontainers that respond to variation of physiological temperature or particular nucleases. Importantly, novel nucleic acids such as aptamers and DNAzymes could be incorporated into this system to construct multifunctional stimulus-responsive devices. Target-directing molecules can also easily be immobilized to the external surface of MSPs to increase the specificity toward cancer cells and minimize toxicity to the surrounding normal tissues.

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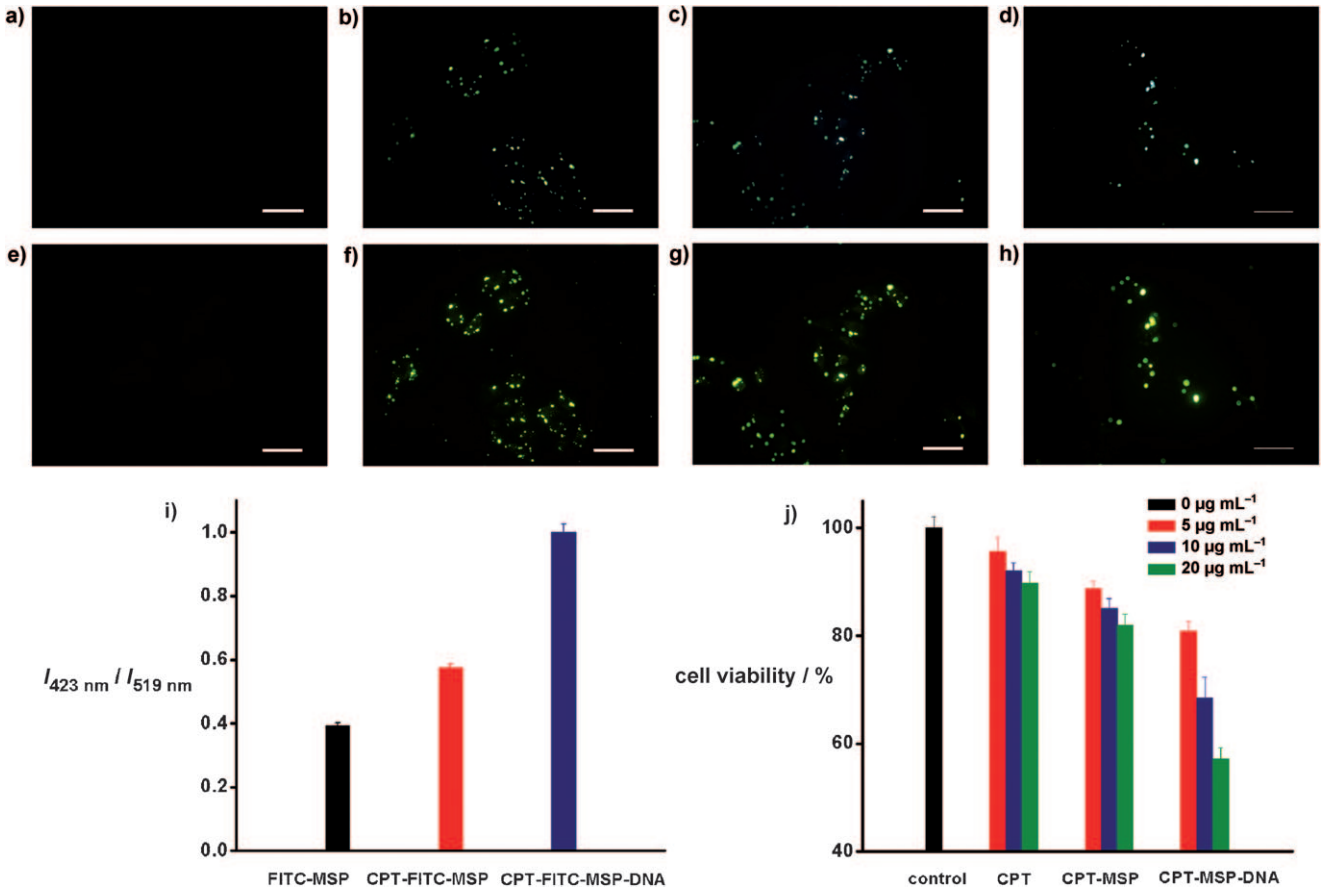

Figure 4. Fluorescence of HepG2 cells excited at 365 nm after incubation with a suspension of a) CPT in PBS, b) FITC-labeled MSPs, c) CPT-loaded FITC-labeled MSPs, and d) CPT-loaded FITC-labeled MSPs-DNA for 3 h. Fluorescence of HepG2 cells excited at 435 nm after incubation with a suspension of e) CPT in PBS, f) FITC-labeled MSPs, g) CPT-loaded FITC-labeled MSPs, and h) CPT-loaded FITC-labeled MSPs-DNA for 3 h. Scale bars: 5 μm. i) Normalized fluorescence ratios ($I_{423\,nm}/I_{519\,nm}$) of HepG2 cells after incubation with a suspension of FITC-labeled MSPs, CPT-loaded FITC-labeled MSPs and CPT-loaded FITC-labeled MSPs-DNA for 3 h. The fluorescence ratio of CPT (423 nm) and fluorescein (519 nm) in each sample correlates with the amount of intracellular CPT. j) Cell viability assay of CPT and CPT-loaded nanoparticles toward HepG2 cells.

Keywords: click chemistry · DNA · drug delivery · mesoporous materials · nanoparticles

[1] a) A. B. Descalzo, R. Martinez-Manez, R. Sancenon, K. Hoffmann, K. Rurack, *Angew. Chem.* **2006**, *118*, 6068; *Angew. Chem. Int. Ed.* **2006**, *45*, 5924; b) S. Saha, K. C. F. Leung, T. D. Nguyen, J. F. Stoddart, J. I. Zink, *Adv. Funct. Mater.* **2007**, *17*, 685; c) I. I. Slowing, J. L. Vivero-Escoto, C. W. Wu, V. S. Y. Lin, *Adv. Drug Delivery Rev.* **2008**, *60*, 1278; d) E. Climent, M. D. Marcos, R. Martinez-Manez, F. Sancenon, J. Soto, K. Rurack, P. Amoros, *Angew. Chem.* **2009**, *121*, 8671; *Angew. Chem. Int. Ed.* **2009**, *48*, 8519.

[2] a) N. K. Mal, M. Fujiwara, Y. Tanaka, *Nature* **2003**, *421*, 350; b) N. G. Liu, D. R. Dunphy, P. Atanassov, S. D. Bunge, Z. Chen, G. P. Lopez, T. J. Boyle, C. J. Brinker, *Nano Lett.* **2004**, *4*, 551; c) R. Liu, X. Zhao, T. Wu, P. Feng, *J. Am. Chem. Soc.* **2008**, *130*, 14418.

[3] 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; b) F. Torney, B. G. Trewyn, V. S. Y. Lin, K. Wang, *Nat. Nanotechnol.* **2007**, *2*, 295; c) J. L. Vivero-Escoto, I. I. Slowing, C. W. Wu, V. S. Y. Lin, *J. Am. Chem. Soc.* **2009**, *131*, 3462.

[4] a) 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; b) T. D. Nguyen, K. C. F. Leung, M. Liong, Y. Liu, J. F. Stoddart, J. I. Zink, *Adv. Funct. Mater.* **2007**, *17*, 2101.

[5] a) R. Hernandez, H. R. Tseng, J. W. Wong, J. F. Stoddart, J. I. Zink, *J. Am. Chem. Soc.* **2004**, *126*, 3370; b) T. D. Nguyen, Y. Liu, S. Saha, K. C. F. Leung, J. F. Stoddart, J. I. Zink, *J. Am. Chem. Soc.* **2007**, *129*, 626.

[6] a) R. Casasús, M. D. Marcos, R. Martinez-Manez, J. V. Ros-Lis, J. Soto, L. A. Villaescusa, P. Amoros, D. Beltran, C. Guillem, J. Latorre, *J. Am. Chem. Soc.* **2004**, *126*, 8612; b) C. Park, K. Oh, S. C. Lee, C. Kim, *Angew. Chem.* **2007**, *119*, 1477; *Angew. Chem. Int. Ed.* **2007**, *46*, 1455; c) S. Angelos, Y. W. Yang, K. Patel, J. F. Stoddart, J. I. Zink, *Angew. Chem.* **2008**, *120*, 2254; *Angew. Chem. Int. Ed.* **2008**, *47*, 2222; d) R. Casasús, E. Climent, M. D. Marcos, R. Martinez-Manez, F. Sancenon, J. Soto, P. Amoros, J. Cano, E. Ruiz, *J. Am. Chem. Soc.* **2008**, *130*, 1903; e) E. Aznar, M. D. Marcos, R. Martinez-Manez, F. Sancenon, J. Soto, P. Amoros, C. Guillem, *J. Am. Chem. Soc.* **2009**, *131*, 6833; f) Q. Fu, G. V. R. Rao, L. K. Ista, Y. Wu, B. P. Andrzejewski, L. A. Sklar, T. L. Ward, G. P. Lopez, *Adv. Mater.* **2003**, *15*, 1262.

[7] a) K. Patel, S. Angelos, W. R. Dichtel, A. Coskun, Y. Yang, J. I. Zink, J. F. Stoddart, *J. Am. Chem. Soc.* **2008**, *130*, 2382; b) A. Schlossbauer, J. Kecht, T. Bein, *Angew. Chem.* **2009**, *121*, 3138; *Angew. Chem. Int. Ed.* **2009**, *48*, 3092; c) A. Bernardos, E. Aznar, M. D. Marcos, R. Martinez-Manez, F. Sancenon, J. Soto, J. M. Barat, P. Amoros, *Angew. Chem.* **2009**, *121*, 5998; *Angew. Chem.*

Int. Ed. **2009**, *48*, 5884; d) P. D. Thornton, A. Heise, *J. Am. Chem. Soc.* **2010**, *132*, 2024.
[8] K. C. F. Leung, T. D. Nguyen, J. F. Stoddart, J. I. Zink, *Chem. Mater.* **2006**, *18*, 5919.
[9] a) Y. Zhu, M. Fujiwara, *Angew. Chem.* **2007**, *119*, 2291; *Angew. Chem. Int. Ed.* **2007**, *46*, 2241; b) D. P. Ferris, Y. Zhao, N. M. Khashab, H. A. Khatib, J. F. Stoddart, J. I. Zink, *J. Am. Chem. Soc.* **2009**, *131*, 1686.
[10] Y. Zhao, B. G. Trewyn, I. I. Slowing, V. S. Y. Lin, *J. Am. Chem. Soc.* **2009**, *131*, 8398.
[11] a) N. C. Seeman, *Mol. Biotechnol.* **2007**, *37*, 246; b) P. Alberti, J. Mergny, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 1569; c) J. Sharma, R. Chhabra, A. Cheng, J. Brownell, Y. Liu, H. Yan, *Science* **2009**, *323*, 112; d) Y. W. Kwon, C. H. Lee, D. H. Choi, J. I. Jin, *J. Mater. Chem.* **2009**, *19*, 1353.
[12] S. Huh, J. W. Wiench, J. C. Yoo, M. Pruski, V. S. Y. Lin, *Chem. Mater.* **2003**, *15*, 4247.
[13] C. D. Hein, X. Liu, D. Wang, *Pharm. Res.* **2008**, *25*, 2216.
[14] a) R. Ranjan, W. J. Brittain, *Macromol. Rapid Commun.* **2008**, *29*, 1104; b) C. Park, H. Kim, S. Kim, C. Kim, *J. Am. Chem. Soc.* **2009**, *131*, 16614; c) C. Park, K. Lee, C. Kim, *Angew. Chem.* **2009**, *121*, 1301; *Angew. Chem. Int. Ed.* **2009**, *48*, 1275.
[15] A. Schlossbauer, D. Schaffert, J. Kecht, E. Wagner, T. Bein, *J. Am. Chem. Soc.* **2008**, *130*, 12558.
[16] E. S. Rangarajan, V. Shankar, *FEMS Microbiol. Rev.* **2001**, *25*, 583.
[17] I. I. Slowing, B. G. Trewyn, V. S. Y. Lin, *J. Am. Chem. Soc.* **2006**, *128*, 14792.
[18] a) J. Lu, M. Liong, J. I. Zink, F. Tamanoi, *Small* **2007**, *3*, 1341; b) J. Lu, E. Choi, F. Tamanoi, J. I. Zink, *Small* **2008**, *4*, 421.
[19] D. A. Giljohann, D. S. Seferos, P. C. Patel, J. E. Millstone, N. L. Rosi, C. A. Mirkin, *Nano Lett.* **2007**, *7*, 3818.

