Host–Guest Systems

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Biotin–Avidin as a Protease-Responsive Cap System for Controlled Guest Release from Colloidal Mesoporous Silica**

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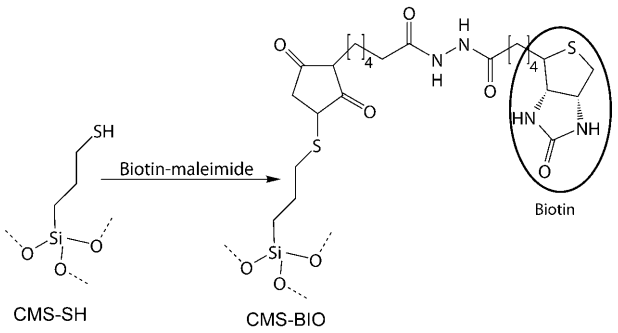
Recently, cap systems that function by stimuli-responsive release mechanisms have been developed for colloidal mesoporous silica (CMS).[1,2] In the design of smart detergents, among other possible applications, the controlled release of molecules such as peptides or antibacterial agents has attracted growing attention.[3,4] CdS nanoparticles, polymers, or large molecules (cyclodextrins and rotaxanes) have been used as cap systems to keep compounds from leaching out of porous hosts and to permit their controlled release.[5,6] Opening stimuli include changes in the pH value or temperature, as well as light, esterase activity, and redox reactions, depending on the system.[5,7] However, many of the existing cap systems still present challenges in terms of their biocompatibility or the toxicity of the capping agents used. One possible solution could be the direct use of native biomolecules, such as proteins, to block the pores of CMS reversibly. It is anticipated that such species would enable high biocompatibility and tailored interactions between the CMS and the locus of activity. Opening of the pore system is possible by removal of the capping system; that is, by the direct cleavage of a link or, as demonstrated in this study, through a decrease in protein bonding interactions by proteolytic hydrolysis.

Until now, the biotin–avidin system has mainly been used in biorecognition,[8] biosensing,[9] and biomedical applications.[10,11] Herein we describe the use of the well-studied biotin–avidin complex as a biomolecule-based, enzyme-responsive cap system for CMS nanoparticles, and demonstrate the operability of this system with the controlled release of fluorescein molecules. Thus, it is possible to design a sophisticated biocompatible cap system with protease-responsive properties by the direct application of an existing biological system. The presence of proteases in modern detergents offers the possibility of a protease-responsive opening mechanism to release sensitive substances that benefit from protective encapsulation.[12]

Recently, we developed a strategy for integrating molecular functionality exclusively into the outer surface shell of CMS in precisely controlled amounts, while leaving the inner pore system unfunctionalized.[13] In the current study, we used this method to design mesoporous nanospheres with protein-coupling sites located exclusively on the outer particle surface. This approach enables the attachment of large proteins without uncontrolled pore clogging inside the mesoporous hosts, and the whole pore volume is available for cargo molecules. For reference experiments, unfunctionalized CMS was synthesized according to our previously reported procedures.[14] Tetraethylorthosilicate (TEOS) was hydrolyzed in a reaction mixture containing cetyltrimethylammonium chloride (CTAC) and triethanolamine (TEA). To obtain CMS selectively functionalized on the outer surface, we applied our previously developed cocondensation approach. The addition of a mixture of TEOS and (3-triethoxysilyl)-1-propanethiol equivalent to 2% of the total silane content 30 min after the generation of the seeds resulted in the formation of mesoporous silica nanoparticles bearing propanethiol moieties exclusively on the outer particle surface (CMS-SH). After 12 h, the template-filled pores of both samples, CMS and CMS-SH, were extracted. The resulting clear suspensions contained particles of around 80 nm in size with BET (Brunauer–Emmett–Teller) surfaces of approximately 1100 $m^2 g^{-1}$ and pore sizes of about 3.8 nm according to nonlocal density functional theory (NLDFT). For TEM pictures of the CMS, see the Supporting Information. The subsequent treatment of CMS-SH in aqueous solution at room temperature with biotin-maleimide (in twofold excess with respect to the thiol groups incorporated in the CMS) resulted in biotinylation of the outer surface of CMS-SH (to give CMS-BIO, Scheme 1).

CMS-BIO was then loaded with fluorescein by stirring CMS-BIO (10 mg) in 25 mL of a 1M solution of fluorescein for



Scheme 1. Attachment of biotin-maleimide to the thiol-functionalized CMS surface.

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1 h. After centrifugation and redispersion in a citrate buffer solution (pH 7), avidin (5 mg) was added to cap the filled pores (CMS-AVI, Figure 1). The closure reaction was performed by stirring at room temperature for 12 h. The resulting

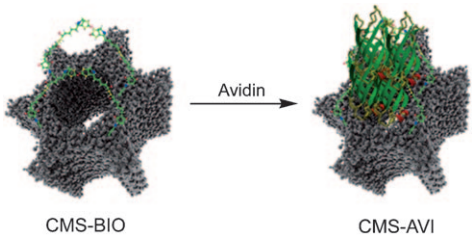

Figure 1. Attachment of avidin caps to the biotinylated CMS surface. The four subunits of avidin can each bind to a biotin moiety attached to the surface.

material was washed three times to remove all dye molecules located outside the closed pores. Unfunctionalized CMS was also loaded with dye as described for CMS-BIO, but only one washing step was performed. The results of the different reaction steps were investigated by IR spectroscopy (Figure 2). The emerging broad absorption band at around

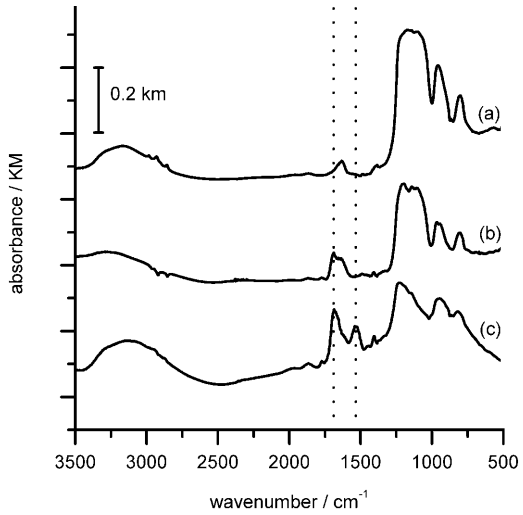

Figure 2. IR spectra of a) CMS-SH, b) CMS-BIO, and c) CMS-AVI. The absorbance is expressed in Kubelka–Munk (KM) units.

1680 cm^{-1} in the sample CMS-BIO can be assigned to various vibrations of the hydrazide and cyclic-urea structures contained within the attached biotin-maleimide molecules (Figure 2b). The typical amide vibrations in the sample CMS-AVI are located at 1530 cm^{-1} and at around 1650 cm^{-1} (Figure 2c). Signals below 1500 cm^{-1} can be mainly attributed to the silica framework. Changes in pore volume and diameter were investigated by nitrogen sorption experiments (Figure 3). For NLDFT calculations, only cumulative pore volumes are specified for pores smaller than 8 nm owing to the considerable textural porosity of CMS samples.

No decrease in pore diameter was observed in the samples CMS, CMS-SH, and CMS-BIO. This result indicates that the

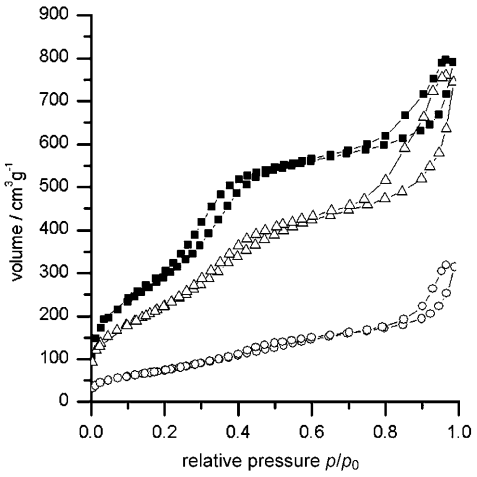

Figure 3. Nitrogen sorption isotherms of the samples CMS-SH (■), CMS-BIO (△), and CMS-AVI (○).

internal surface of the mesoporous particles remains unfunctionalized (Table 1). The corresponding pore-size-distribution graphs can be found in the Supporting Information.

Table 1: NLDFT pore diameters and pore volumes for the CMS samples synthesized.

	CMS	CMS-SH	CMS-BIO	CMS-AVI
pore diameter [nm]	3.9	3.8	3.8	–
pore volume [cm^{3}g^{-1}]	0.8	0.8	0.6	0.19

The decrease in surface area and pore volume by approximately 30% in the sample CMS-BIO is attributed to partial pore blocking due to the large organic moieties on the outer shell of the CMS. In the case of CMS-AVI, a striking, almost complete elimination of nitrogen-accessible mesopore volume was observed as a result of pore blocking of the mesopores by the large proteins. We take this observation as evidence of a very effective pore closure by the attached avidin.

Measurements of zeta potential were made to show the effect of the different functionalization steps on the surface charge (see the Supporting Information). The enzyme-responsive release properties were investigated by fluorescence spectroscopy. An aqueous suspension containing 2 mg of either CMS-AVI or loaded unfunctionalized CMS was transferred into a specially designed container, which could be closed by a holey lid lined with a dialysis membrane. This custom-made system fits on the opening of a fluorescence cuvette (see the Supporting Information).

Whereas the colloidal particles are too large to diffuse through the dialysis membrane, fluorescein can enter the free cuvette volume readily and be observed by fluorescence spectroscopy at 37 °C. In a reference experiment, it was shown that the dialysis membrane used, with a molecular-weight cutoff of 16000 g mol^{-1}, does not act as a diffusion barrier for fluorescein (see the Supporting Information). Fluorescein

was excited at 490 nm, which led to a fluorescence emission maximum at 511 nm. In another reference experiment, the unfunctionalized CMS host released the entire amount of loaded dye within minutes (Figure 4, ■). The concentration of fluorescein inside the cuvette remained stable after 30 min.

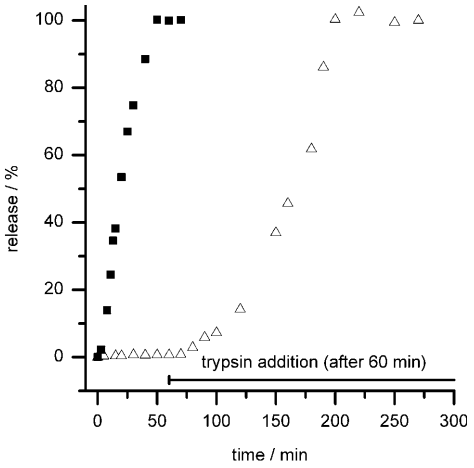

Figure 4. Protease-responsive release curve for CMS-AVI (△), and release from unfunctionalized CMS (■).

This concentration was attributed to a relative release ratio of 100%. The attached avidin in the sample CMS-AVI prevented the loaded fluorescein from escaping from the pore system. After 60 min, no significant release of fluorescein was observed, which indicates that the new closure mechanism is highly efficient.

A very different result was obtained after the addition of the protease trypsin (1 mg) to the colloidal suspension. In an earlier study, trypsin had been used to hydrolyze avidin to obtain information about its amino acid sequence.[15] In our case, the proteolytic digestion of avidin enables the loaded dye to escape from its host. The concentration of released fluorescein increased shortly after the addition of trypsin (Figure 4, △). After 4 h, no increase in the concentration of released fluorescein was observed, a result that indicated the complete release of the loaded dye.

Interestingly, the enzyme-responsive release curve features a fairly slow release for the first hour after the addition of trypsin. This result can be explained by the tryptic-hydrolysis process, in which an increasing number of caps are cleaved. After 1 h, the capping proteins are effectively digested, which leads to a faster release of the guest molecules. After 140 min following the addition of the protease, the observed amount of fluorescein remained stable (100%). The absolute amounts of released dye were determined by UV/Vis spectroscopy (see the Supporting Information). These data clearly demonstrate that we were able to close the pore system of CMS with the avidin–biotin system, and to release the loaded molecules subsequently by enzymatic hydrolysis of the caps.

We carried out a complementary thermoresponsive release experiment to compare the efficiency of the different cap-opening methods. In this case, the attached protein caps were opened by denaturation. Therefore, they were opened immediately and simultaneously. An increase in the temperature to 90 °C weakened the affinity of avidin for the biotinylated surface and led to the fast and fairly linear release of the loaded fluorescein molecules (Figure 5). In contrast to the enzyme-responsive opening mechanism, thermoresponsive release occurs directly after stimulation (temperature increase).

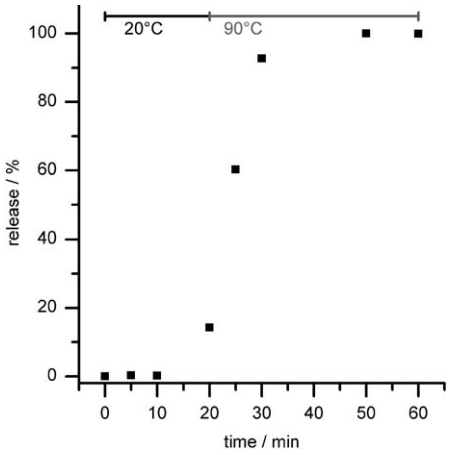

Figure 5. Thermoresponsive fluorescein release from the sample CMS-AVI.

In summary, we have presented a biomolecule-based enzyme-responsive cap system for mesoporous silica. We have shown that the attachment of avidin to the outer surface of CMS can prevent the uncontrolled leaching of incorporated guest molecules. The tight closure of the pores can be explained by the structure of the avidin–biotin complex. The four subunits of avidin (molecular size: $4.5 \times 5.5 \times 6\ nm^3$)[16] can each bind to a biotin molecule; thus, a strong interaction between the avidin and the biotin-covered CMS surface results. We have demonstrated an opening mechanism based on the controlled enzymatic hydrolysis of the attached protein avidin. The advantage of this system lies in the use of native biomolecules. Thus, the creation of toxic or carcinogenic species can be avoided. This approach offers new possibilities for the application of mesoporous hosts in the fields of detergent design and drug delivery.

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[1] S. Giri, B. G. Trewyn, V. S.-Y. Lin, *Nanomedicine* **2007**, *2*, 99.
[2] I. I. Slowing, B. G. Trewyn, S. Giri, V. S.-Y. Lin, *Adv. Funct. Mater.* **2007**, *17*, 1225.
[3] B. C. Dave, K. Deshpande, M. S. Gebert, J. C. McAuliffe, *Adv. Mater.* **2006**, *18*, 2009.
[4] B. G. Trewyn, C. M. Whitman, V. S.-Y. Lin, *Nano Lett.* **2004**, *4*, 2139.

[5] B. G. Trewyn, S. Giri, I. I. Slowing, V. S.-Y. Lin, *Chem. Commun.* **2007**, 3236.
[6] I. Izquierdo-Barba, M. Manzano, M. Colilla, M. Vallet-Regi, *Key Eng. Mater.* **2008**, *377*, 133.
[7] 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.
[8] D. Prosperi, C. Morasso, P. Tortora, D. Monti, T. Bellini, *ChemBioChem* **2007**, *8*, 1021.
[9] J.-I. Anzai, *Chem. Sens.* **2002**, *18*, 55.
[10] B. Barat, A. M. Wu, *Biomol. Eng.* **2007**, *24*, 283.
[11] N. Kojima, T. Matsuo, Y. Sakai, *Biomaterials* **2006**, *27*, 4904.
[12] K.-H. Maurer, *Curr. Opin. Biotechnol.* **2004**, *15*, 330.
[13] J. Kecht, A. Schlossbauer, T. Bein, *Chem. Mater.* **2008**, *20*, 7207.
[14] K. Möller, J. Kobler, T. Bein, *Adv. Funct. Mater.* **2007**, *17*, 605.
[15] T.-S. Huang, R. J. DeLange, *J. Biol. Chem.* **1971**, *246*, 686.
[16] L. Pugliese, A. Coda, M. Malcovati, M. Bolognesi, *J. Mol. Biol.* **1993**, *231*, 698.

