Functional Nanoparticles

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Controlled Delivery Using Oligonucleotide-Capped Mesoporous Silica Nanoparticles**

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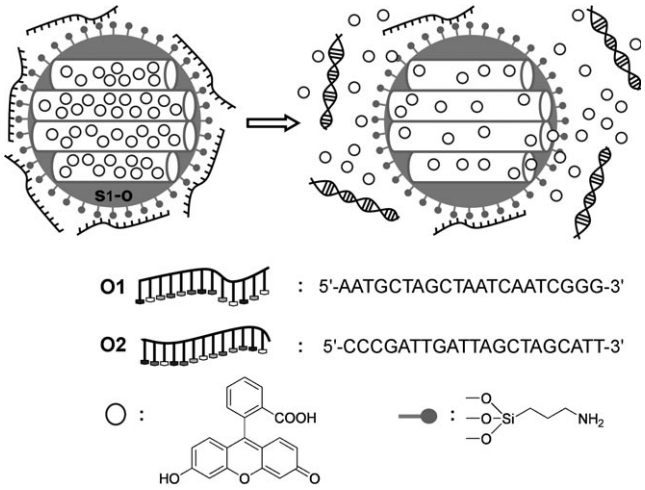
In memory of María Dolores Climent Morató

The design of delivery systems with "molecular locks" able to selectively release entrapped guests in the presence of target triggers has attracted great attention recently.[1] As an alternative to traditional polymer-based delivery systems, mesoporous silica supports show unique properties such as a large load capacity, biocompatibility, and potential for the development of gated supports for on-command delivery applications.[2] Recently, mesoporous-silica-based systems displaying controlled release have been reported relying on changes in pH, redox potential, and light for uncapping the pores.[3] However, the use of mesoporous silica supports equipped with gatelike scaffoldings is still an incipient area of research. In particular, despite some recent reported gated mesoporous silica supports that can be uncapped using antigens[4] or enzymes,[5] there is an almost complete lack of mesoporous-silica-based devices designed to trigger cargo release involving biomolecules. Within this context, our interest in the development of gated materials[6] motivated us to explore the possible design of new "bio-gates" able to respond selectively to "key" molecules. We focused our attention on the use of nucleotides.

The proposed paradigm is represented in Scheme 1. In this work nanoparticles (ca. 100 nm) of mesoporous MCM-41 have been selected as the inorganic scaffold. The MCM-41 support is first loaded with a suitable guest (fluorescein), and then the external surface is functionalized with 3-aminopropyltriethoxysilane (APTS) to give the solid **S1**. Aminopropyl groups are partially charged at neutral pH in water and will interact with negatively charged oligonucleotides, resulting in the closing of the mesopores. The opening protocol will be expected to occur by a highly effective displacement reaction in the presence a target complementary strand; this will result in hybridization of the two oligonucleotides, the uncapping of the pores, and release of the entrapped cargo.



Scheme 1. Representation of the gated material **S1** functionalized with 3-aminopropyltriethoxysilane and capped with a single-stranded oligonucleotide (**O1**). The delivery of the entrapped guest (fluorescein) is selectively accomplished in the presence of the complementary oligonucleotide (**O2**). The sequence of the oligonucleotides **O1** and **O2** is shown.

The mesoporous solid **S1** containing fluorescein in the pore voids and functionalized on the external surface with APTS groups was characterized following standard procedures (see the Supporting Information). The powder X-ray diffraction (XRD) pattern of siliceous MCM-41 nanoparticles as synthesized (Figure 1, curve a) shows four low-angle reflections typical of a hexagonal array which can be indexed as (100), (110), (200), and (210) Bragg peaks. A significant displacement of the (100) peak in the XRD pattern of the MCM-41 calcined nanoparticles is evident in curve b. Finally, curve c corresponds to the XRD pattern of **S1**. The (100), (110), and (200) peaks are clearly observed strongly suggesting that the dye loading and further functionalization with

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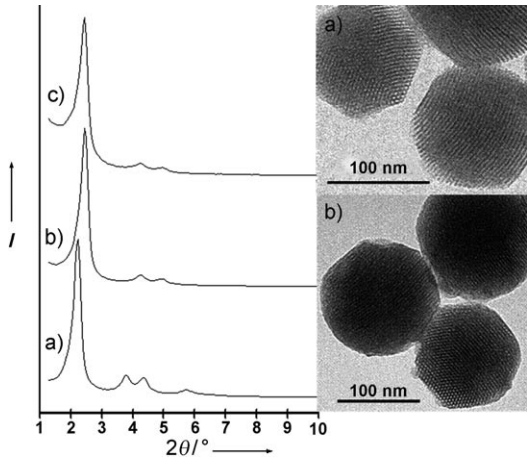

Figure 1. Left: powder X-ray patterns of the solids a) MCM-41 as synthesized, b) calcined MCM-41, and c) solid **S1** containing the dye fluorescein and 3-aminopropyltriethoxysilane. Right: TEM images of calcined MCM-41 sample (a) and solid **S1** (b) showing the typical porosity of the MCM-41 mesoporous matrix.

APTS have not damaged the three-dimensional mesoporous MCM-41 scaffolding.

The N_2 adsorption–desorption isotherms of the calcined MCM-41 nanoparticles show an adsorption step at an intermediate P/P_0 value (0.1–0.3) typical of this type of solid (see the Supporting Information). A pore volume of 0.61 $cm^3 g^{-1}$ was calculated by using the BJH model on the adsorption branch of the isotherm. The application of the BET model resulted in a value for the total specific surface of 825 $m^2 g^{-1}$. From the XRD, porosimetry, and TEM studies a pore diameter of 2.41 nm was determined. The N_2 adsorption–desorption isotherm of **S1** is typical of mesoporous systems with filled mesopores, and a significant decrease in the N_2 volume adsorbed and surface area (245 $m^2 g^{-1}$) was observed.

For the preparation of the gated material **S1-O1**, 500 μg of **S1** was suspended in 500 μL of the hybridization buffer (20 mM Tris-HCl, 37.5 mM $MgCl_2$, pH 7.5)[7] containing the oligonucleotide **O1** (5′-AATGCTAGCTAATCAATCGGG-3′) for a final concentration of 0.026 mmol g^{-1} SiO_2. The final **S1-O1** solid was isolated by centrifugation and washed with hybridization buffer (2 × 1 mL) to eliminate the residual fluorescein dye and the free oligonucleotide **O1.**

The quantities of dye and APTS on solid **S1** were determined by elemental analysis and thermogravimetric studies to be 0.078 and 1.98 mmol g^{-1} SiO_2, respectively. Additionally, the amount of oligonucleotide **O1** in solid **S1-O1** was determined by the use of the oligonucleotide **O1′** (5′-AATGCTAGCTAATCAATCGGG-Cy5-3′), which is similar to **O1** but marked with a Cy5 dye; the decrease of the fluorescence emission of **O1′** in solution was measured during the capping process. A content of 0.022 mmol of oligonucleotide per gram of SiO_2 was found.

To investigate the gating properties of **S1-O1**, 500 μg of this solid was suspended in 1 mL of the hybridization buffer, and the suspension was divided into two fractions. A 0.25 mL aliquot of the hybridization buffer was added to the first fraction. The second fraction was treated with 0.25 mL of hybridization buffer containing the target complementary oligonucleotide **O2** (5′-CCCGATTGATTAGCTAGCATT-3′) in a concentration of 0.022 mmol g^{-1} SiO_2. In both cases the suspensions were stirred for 3 h at 37 °C. The gating mechanism was monitored through the measurement of the fluorescence emission of the released fluorescein. The delivery profile of fluorescein in the presence and absence of the target oligonucleotide is displayed in Figure 2. As can

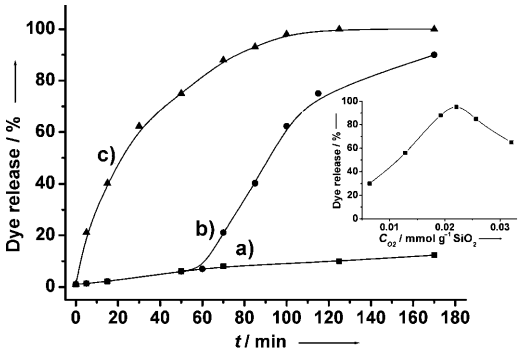

Figure 2. Release of fluorescein from solid **S1-O1** in the absence (a) and in the presence (c) of oligonucleotide **O2** (0.022 mmol g^{-1} SiO_2) in hybridization buffer (20 mM Tris-HCl, 37.5 mM $MgCl_2$, pH 7.5). Curve b shows the release profile of fluorescein from **S1-O1** in hybridization buffer until $t = 60$ min when 0.022 mmol of **O2** g^{-1} SiO_2 was added. Inset: Percentage of released fluorescein dye from **S1-O1** as a function of the concentration of **O2** after 70 min of reaction.

be seen, solid **S1-O1** is tightly capped and shows a negligible release of fluorescein (curve a). In contrast, the presence of the complementary oligonucleotide **O2** induced the hybridization between **O1** and **O2**, the opening of the pores, and delivery of the dye (curve c). Curve c also shows that within 90 min more than 95 % of the fluorescein had been released (48 % of the total adsorbed fluorescein in solid **S1-O1**). Additionally, curve b displays the release profile of the fluorescein from **S1-O1** up until $t = 60$ min, when suddenly **O2** at a concentration of 0.022 mmol g^{-1} SiO_2 was added to the solution.

The delivery of dye from **S1-O1** was also studied as a function of the amount of the molecular trigger (oligonucleotide **O2**, see inset in Figure 2). It can be seen that the delivery of the cargo is proportional to the **O2** concentration. The maximum release was observed at an **O2** concentration of 0.022 mmol g^{-1} SiO_2; at higher concentrations the delivery was partially inhibited, most likely because excess **O2** was adsorbed onto the surface of the solid, inducing partial pore capping.

The uncapping mechanism (Scheme 1) was also confirmed by using solid **S1-O1′** in which the pores had been capped with the Cy5-marked oligonucleotide **O1′**. Solid **S1-O1′** shows a blue coloration due to the Cy5 dye. After addition of the complementary single-strand oligonucleotide **O2** the solid became colorless, and the typical emission band of Cy5 centered at 670 nm (excitation at 649 nm) was

observed in the aqueous phase indicating the release to the solution of the **O1′-O2** fragment, and excluding the possibility that double-stranded **O1′-O2** system remained adsorbed on the silica surface.

In order to investigate selectivity in the opening protocol, dye delivery from **S1-O1** was tested in the presence of other oligonucleotides similar to **O2**; that is, **O3** (5′-CCCGATTGATTCTCTAGCATT-3′), a two-base mismatch sequence, and **O4** (5′-CCCGATTGATTGGCTAGCATT-3′), an oligonucleotide with a single-base mismatch sequence. The uncapping ability of **O2**, **O3**, and **O4** (at a concentration of 0.022 mmol g^{-1} SiO_2) as a function of time is displayed in Figure 3. The results show that only a remarkable release of

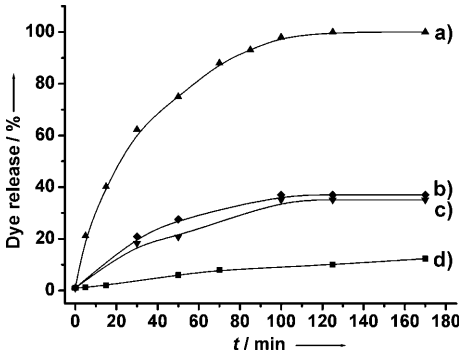

Figure 3. Emission intensity of released fluorescein dye from solid **S1-O1** in hybridization buffer (20 mM Tris-HCl, 37.5 mM $MgCl_2$, pH 7.5) in the presence of oligonucleotide **O2** (a), **O4** (b), and **O3** (c) at concentrations of 0.022 mmol g^{-1} SiO_2, and in the absence of oligonucleotide (d).

dye was observed with **O2** (full hybridization with complementary **O1**), whereas the presence of **O3** and **O4** induced a poor uncapping of the pores. Moreover, we observed that the presence of random oligonucleotides did not induce dye delivery.

In summary, we have demonstrated that the use of oligonucleotides as caps on the surface of mesoporous supports is a suitable method for the preparation of "biogated" delivery systems that can be selectively opened in the presence of specific targets (i.e. the complementary oligonucleotide). The possibility of preparing similar systems on different supports and to select and easily synthesize tailor-made oligonucleotides makes this approach of interest in a wide range of timely research fields such as delivery protocols and diagnosis (recognition of certain oligonucleotide chains), and in the design of "keypad-lock-type" systems, which can be activated (opened) only with the correct combination of nucleotides.

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[1] a) A. B. Descalzo, R. Martínez-Máñez, F. Sancenón, K. Hoffmann, K. Rurack, *Angew. Chem.* **2006**, *118*, 6068 – 6093; *Angew. Chem. Int. Ed.* **2006**, *45*, 5924 – 5948; b) I. I. Slowing, J. L. Vivero-Escoto, C. W. Wu, V. S.-Y Lin, *Adv. Drug Delivery Rev.* **2008**, *60*, 1278 – 1288; c) E. Aznar, R. Martínez-Máñez, F. Sancenón, *Expert Opin. Drug Delivery* **2009**, *6*, 643 – 655; d) 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) M. Vallet-Regí, F. Balas, D. Arcos, *Angew. Chem.* **2007**, *119*, 7692 – 7703; *Angew. Chem. Int. Ed.* **2007**, *46*, 7548 – 7558.

[2] a) A. P. Wight, M. E. Davis, *Chem. Rev.* **2002**, *102*, 3589 – 3614; b) G. Kickelbick, *Angew. Chem.* **2004**, *116*, 3164 – 3166; *Angew. Chem. Int. Ed.* **2004**, *43*, 3102 – 3104; c) A. Stein, *Adv. Mater.* **2003**, *15*, 763 – 775.

[3] For recent examples see for instance: a) R. Liu, Y. Zhang, X. Zhao, A. Agarwal, L. J. Mueller, P. Feng, *J. Am. Chem. Soc.* **2010**, *132*, 1500 – 1501; b) Y. Zhu, H. Liu, F. Li, Q. Ruan, H. Wang, M. Fujiwara, L. Wang, G. Q. Lu, *J. Am. Chem. Soc.* **2010**, *132*, 1450 – 1451; c) R. Mortera, J. Vivero-Escoto, I. I. Slowing, E. Garrone, B. Onida, V. S.-Y Lin, *Chem. Commun.* **2009**, 3219 – 3221; d) Y. Zhao, B. G. Trewyn, I. I. Slowing, V. S.-Y Lin, *J. Am. Chem. Soc.* **2009**, *131*, 8398 – 8400; e) R. Liu, X. Zhao, T. Wu, P. Feng, *J. Am. Chem. Soc.* **2008**, *130*, 14418 – 14419; f) F. Torney, B. G. Trewyn, V. S.-Y. Lin, K. Wang, *Nat. Nanotechnol.* **2007**, *2*, 295 – 300; g) I. I. Slowing, B. G. Trewyn, S. Giri, V. S.-Y Lin, *Adv. Funct. Mater.* **2007**, *17*, 1225 – 1236; h) T. D. Nguyen, Y. Liu, S. Saha, K. C.-F. Leung, J. F. Stoddart, J. I. Zink, *J. Am. Chem. Soc.* **2007**, *129*, 626 – 634; i) T. D. Nguyen, K. C. F. Leung, M. Liong, Y. Liu, J. F. Stoddart, J. I. Zink, *Adv. Funct. Mater.* **2007**, *17*, 2101 – 2110.

[4] E. Climent, A. Bernardos, R. Martínez-Máñez, A. Maquieira, M. D. Marcos, N. Pastor-Navarro, R. Puchades, F. Sancenón, J. Soto, P. Amorós, *J. Am. Chem. Soc.* **2009**, *131*, 14075 – 14080.

[5] a) 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; b) A. Schlossbauer, J. Kecht, T. Bein, *Angew. Chem.* **2009**, *121*, 3138 – 3141; *Angew. Chem. Int. Ed.* **2009**, *48*, 3092 – 3095; c) A. Bernardos, E. Aznar, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, J. M. Barat, P. Amorós, *Angew. Chem.* **2009**, *121*, 5998 – 6001; *Angew. Chem. Int. Ed.* **2009**, *48*, 5884 – 5887; d) C. Park, H. Kim, S. Kim, C. Kim, *J. Am. Chem. Soc.* **2009**, *131*, 16614 – 16615; e) P. D. Thornton, A. Heise, *J. Am. Chem. Soc.* **2010**, *132*, 2024 – 2028.

[6] See for instance: a) R. Casasús, E. Climent, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, P. Amorós, J. Cano, E. Ruiz, *J. Am. Chem. Soc.* **2008**, *130*, 1903 – 1917; b) C. Coll, R. Casasús, E. Aznar, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, P. Amorós, *Chem. Commun.* **2007**, 1957 – 1959; c) R. Casasús, E. Aznar, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, P. Amorós, *Angew. Chem.* **2006**, *118*, 6813 – 6816; *Angew. Chem. Int. Ed.* **2006**, *45*, 6661 – 6664; d) E. Aznar, C. Coll, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, P. Amorós, J. Cano, E. Ruiz, *Chem. Eur. J.* **2009**, *15*, 6877 – 6888.

[7] L. R. Hilliard, X. Zhao, W. Tan, *Anal. Chim. Acta* **2002**, *470*, 51 – 56.

