Molecular Gates

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Finely Tuned Temperature-Controlled Cargo Release Using Paraffin-Capped Mesoporous Silica Nanoparticles**

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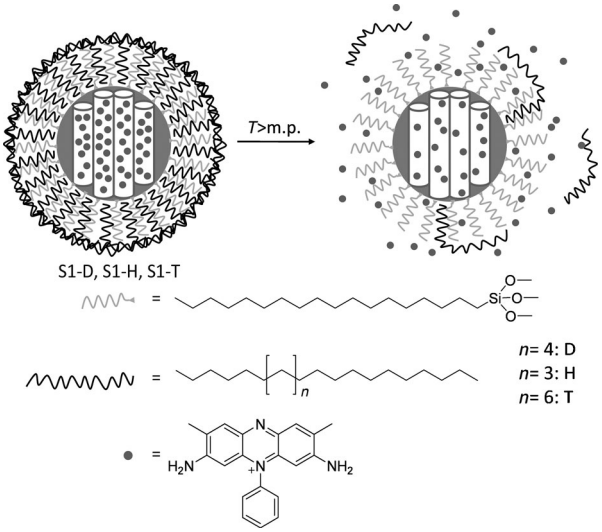
Stimuli-responsive capped mesoporous materials have been demonstrated to be fascinating vehicles for the storage and controlled release of entrapped guests.[1] Chemical triggers such as the presence of anions,[2] small redox molecules,[3] antigens,[4] oligonucleotides,[5] and enzymes[6] have been used to develop gated materials with controlled-release features, but also physical stimuli such as light[7] and temperature[8] have attracted attention thanks to the possibility of remote activation. To date, temperature-controlled release of molecules from pore voids of mesoporous materials has mainly been achieved using a thermosensitive poly(*N*-isopropyl acrylamide) (PNIPAAm) derivative.[8a–g] More recently, Bein et al. described a molecular valve that releases entrapped fluorescein upon heating to the specific melting temperature of double-stranded DNA sequences that are attached to the pore openings of mesoporous nanoparticles.[8h] Despite these examples, temperature-controlled release of molecules from mesoporous materials is still in its infancy, and there are no reported examples where the release temperature could be easily customized depending on target requirements.

Taking into account these concepts and being aware of the promising features of silica mesoporous supports (SMPS) as containers,[9] we focused on the development of new tailor-made temperature-responsive gated mesoporous materials.

The expected performance of the new gated material (Scheme 1) is based on the use of paraffins as capping molecules that can be melted at a defined temperature. Herein, silica mesoporous nanoparticles (ca. 100–150 nm) have been selected as the inorganic scaffold. The mesoporous MCM-41 support was loaded with a suitable guest (Safranine O), and the external surface was functionalized with octadecyltrimethoxysilane to give solid S1. These alkyl chains are not able to close the pores but are suitable to interact with paraffins (see below) through London forces; the paraffins set up a hydrophobic layer that could block the pores and inhibit guest release. An increase of the temperature above the paraffin melting point would result in melting of the paraffin and the subsequent uncapping of the pores.



Scheme 1. Representation of the gated materials S1-D, S1-H, and S1-T functionalized with octadecyltrimethoxysilane and capped with paraffin. The delivery of the entrapped guest (Safranine O) is triggered when temperature rises above the paraffin melting point.

Solid S1 was characterized using standard procedures (see the Supporting Information). The X-ray diffraction pattern of S1 (Figure 1) indicates that the loading process with the dye and the further functionalization with octadecyltrimethoxysilane have not modified the structure of the mesoporous scaffold, which can be concluded from the presence of the (100) diffraction peak characteristic of mesoporous materials.

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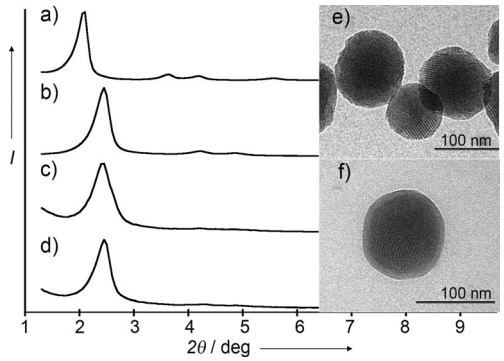

Figure 1. a–d) Powder X-ray diffraction patterns of a) MCM-41 as synthesized, b) calcined MCM-41, c) S1, and d) S1-D. e, f) TEM images of e) calcined MCM-41 and f) S1-D showing the typical porosity of the MCM-41 mesoporous matrix and the paraffin coating.

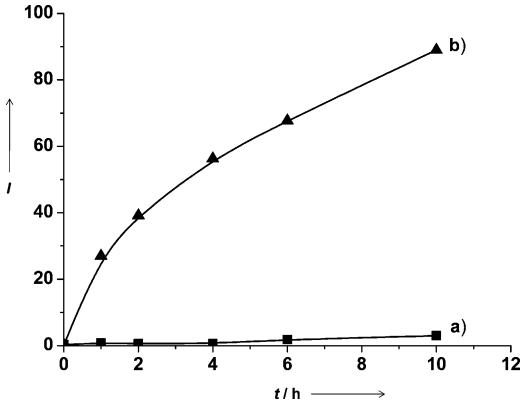

Figure 2. Release of Safranine O from solid S1-D at a) 40 °C and b) 44 °C, monitored by the fluorescence emission intensity *I* at 585 nm ($\lambda_{ex} = 520$ nm).

Moreover, elemental analysis and thermogravimetry indicate that solid S1 contains 0.44 mmol dye per gram SiO_2 and 0.76 mmol octadecyl chains per gram SiO_2.

For the preparation of gated material S1-D, the paraffin docosane (D) was selected (melting point 42 °C). Solid S1 (150 mg) was dispersed in hexane (120 mL) and sonicated for 30 min. Then, D (1.5 g) was added to the suspension (see the Supporting Information for details). The final S1-D solid was isolated by centrifugation and dried under vacuum. This synthetic procedure assures that the final hybrid solid contains Safranine O inside the mesopores and the corresponding paraffin as a coating surrounding the nanoparticle. Solid S1-D was analyzed by using thermogravimetric analysis and was found to contain 0.42 mmol Safranine O and 0.15 mmol paraffin D per gram SiO_2. Figure 1 shows TEM images of calcined MCM-41 and S1-D. For the latter, the paraffin coating can be clearly observed.

To investigate the gating properties of the solid, S1-D was suspended in a thermostatized vase containing deionized water (see the Supporting Information), and the suspension was stirred at 40 °C. The same procedure was repeated with S1-D at 44 °C. The fluorescence emission at 585 nm ($\lambda_{ex} =$ 520 nm) of the released Safranine O at the two temperatures is displayed in Figure 2 as a function of time. At 40 °C solid S1-D was tightly capped and showed a negligible release of the dye (Figure 2 curve a). In contrast, when the temperature was adjusted to 44 °C, the entrapped Safranine O probe escaped from the pore voids as a result of a temperature-dependent uncapping event (Figure 2 curve b). Quantitatively, the release after one hour at 44 °C was 30 times higher than at 40 °C and more than 100 times higher after four hours. Further experiments showed that 36 % of the entrapped dye was released after ten hours at 44 °C.

In a second step, the delivery from S1 materials was studied as a function of the melting point of the temperature-responsive layer. For these experiments the paraffins heneicosane (H, melting point 39 °C) and tetracosane (T, melting point 49 °C) were selected. The corresponding solids S1-H and S1-T were prepared by the same procedure as that used for S1-D. The new solids showed Safranine O release profiles similar to that of S1-D (see Figures SI-1 and SI-3 in the Supporting Information). Furthermore, the temperature-dependent performance of the hybrid materials was evaluated by suspending the corresponding solid in deionized water at a given temperature for two hours (Figure 3). After this time, the suspensions were filtered and the release of Safranine O was determined.

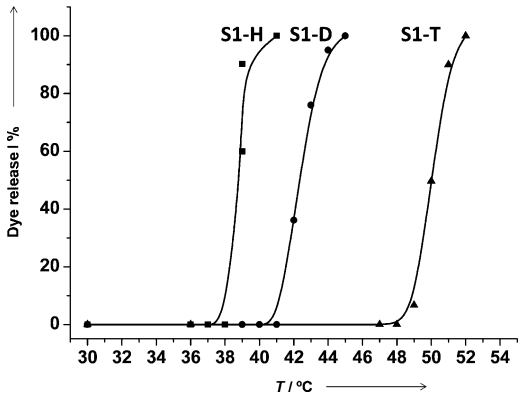

Figure 3. Release profile of solids S1-H, S1-D, and S1-T at different temperatures. Release data were normalized to the maximum delivery obtained for each solid.

The results shown in Figure 3 confirmed how an appropriate paraffin selection makes it possible to fine-tune the triggering event. In fact, the temperatures that were needed to release 50 % (TR50) of the entrapped probe, were 39, 42 and 50 °C for solids S1-H, S1-D, and S1-T, respectively. It is remarkable that solid S1-T showed a negligible release below 49 °C although at such high temperatures diffusion processes could become relevant.

These hybrid nanoparticles can have biological applications as nanodevices for intracellular temperature-controlled release of molecules. To study this possibility, solid S1-H (TR50 39 °C) was initially evaluated in human cancer HeLa cells (see the Supporting Information). The cells were incubated in duplicate in the presence of S1-H for one hour; excess solid S1-H was removed, and the cells were placed in fresh medium for three additional hours to allow complete internalization of the nanoparticles. Subsequently,

one cellular sample set was incubated for three hours at 42 °C to induce melting of the solid S1-H covering, while the second was kept at 37 °C. Twelve hours later, the release of Safranine O was analyzed by confocal microscopy. Figure 4 shows representative images of HeLa cells treated with solid S1-H at 37 °C (Figure 4a) and at 42 °C (Figure 4b). The

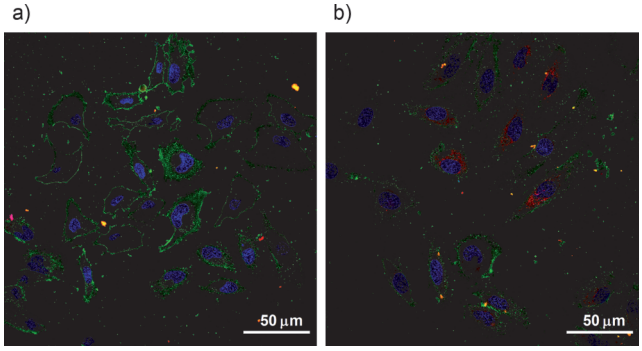

Figure 4. Confocal microscopy images corresponding to HeLa cells treated with solid S1-H (200 μg mL^{-1}) and incubated at a) 37 °C and b) 42 °C. The cellular internalization of S1-H was monitored by Safranine O fluorescence (red) in the presence of DNA marker Hoechst 33342 (blue) and the plasma membrane marker WGA Alexa Fluor 647 (green).

Safranine O probe (in red) was found spread in the cell cytoplasm in cells incubated at 42 °C, but not in those cells incubated at 37 °C, owing to the temperature-dependent uncapping of the internalized solid S1-H.

To demonstrate the possible application of these nanoparticles as temperature-dependent drug carriers for on-command delivery inside cells, a derivative of S1-H was prepared that contained the chemotherapeutic agent doxorubicin (DOX) instead of Safranine O. The release profile and performance of S1-H-DOX were similar to solids loaded with Safranine O (see Figure SI-4 in the Supporting Information). Early stages of S1-H-DOX-induced cell death can be traced by using Annexin V (Ann V) as a marker (see the Supporting Information) for exposed phosphatidylserine at the cell surface. Late stages of cell death are characterized by an increased cell-membrane permeabilization, which allows the incorporation of nuclear markers such as 4′,6-diamidino-2-phenylindole (DAPI). HeLa cells were treated with this new nanoparticle S1-H-DOX as described before. At 37 °C both control and S1-H-DOX-treated cells showed characteristics of healthy cells as determined by flow cytometry (negative for both Ann V and DAPI, see Figure 5). However, confocal images of cells that were treated with S1-H-DOX and incubated at 42 °C showed morphological features such as cell detachment, reduction of cellular volume, and cellular shrinkage. Moreover, at 42 °C, 60 % of the cells treated with S1-H-DOX, but not the control cells, showed characteristics of DOX-induced cell death, and the analysis suggested that close to 40 % of the cells were developing the cell death program (Ann V positive, DAPI negative), while close to 20 % of the cells (DAPI positive) were at the late stages of such a program. These results confirmed the temperature-dependent release of DOX in cells.

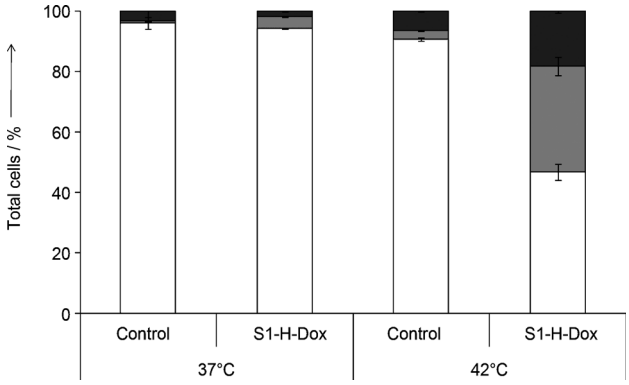

Figure 5. Cell viability studied by flow cytometry. HeLa cells were treated with S1-H-DOX as described for S1-H and incubated at 37 or 42 °C. Control cells were left untreated. Then, DAPI and Ann V stainings were performed to determine cell viability and cell death induction, respectively. Percentage of dead cells (black), cells undergoing cell death (gray), and healthy cells (white) are depicted after 18 h of treatment. Two independent experiments were performed. Data are reported as (mean ± standard error).

In short, we reported herein a new family of temperature-responsive hybrid nanoscopic solids that are uncapped at a given temperature. The materials were prepared using MCM-41 supports functionalized with octadecyltrimethoxysilane and paraffins, which are able to form a hydrophobic layer that inhibits cargo release. An increase of the temperature above the paraffin melting point results in the release of the entrapped guest. The finely tuned tailor-made temperature-triggered delivery achieved in these capped systems and the possibility to select a wide range of paraffins with specific melting points over a wide range of temperatures make these solids suitable for applications that demand zero release before stimulus implementation and render them important for delivery applications triggered by selected global or local temperature changes.

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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) B. G. Trewyn, I. I. Slowing, S. Giri, H. T. Chen, V. S.-Y. Lin, *Acc. Chem. Res.* **2007**, *40*, 846 – 853; c) I. I. Slowing, J. L. Vivero-Escoto, C. W. Wu, V. S.-Y. Lin, *Adv. Drug Delivery Rev.* **2008**, *60*, 1278 – 1288; d) E. Aznar, R. Martínez-Máñez, F. Sancenón, *Expert Opin. Drug Delivery* **2009**, *6*, 643 – 655; e) 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.

[2] 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.

[3] a) F. Torney, B. G. Trewyn, V. S.-Y. Lin, K. Wang, *Nat. Nanotechnol.* **2007**, *2*, 295 – 300; b) D. R. Radu, C.-Y Lai, K. Jeftinija, E. W. Rowe, S. Jeftinija, V. S.-Y. Lin, *J. Am. Chem. Soc.* **2004**, *126*, 13216 – 13217; c) S. Giri, B. G. Trewyn, M. P. Stellmaker, V. S.-Y. Lin, *Angew. Chem.* **2005**, *117*, 5166 – 5172; *Angew. Chem. Int. Ed.* **2005**, *44*, 5038 – 5044; d) I. I. Slowing, B. G. Trewyn, V. S.-Y. Lin, *J. Am. Chem. Soc.* **2007**, *129*, 8845 – 8849; e) I. I. Slowing, B. G. Trewyn, S. Giri, V. S.-Y. Lin, *Adv. Funct. Mater.* **2007**, *17*, 1225 – 1236; f) R. Mortera, J. Vivero-Escoto, I. I. Slowing, E. Garrone, B. Onida, V. S.-Y. Lin, *Chem. Commun.* **2009**, 3219 – 3221; g) Y. Zhao, B. G. Trewyn, I. I. Slowing, V. S.-Y. Lin, *J. Am. Chem. Soc.* **2009**, *131*, 8398 – 8400; h) R. Hernandez, H.-R. Tseng, J. W. Wong, J. F. Stoddart, J. I. Zink, *J. Am. Chem. Soc.* **2004**, *126*, 3370 – 3371; i) T. D. Nguyen, H.-R. Tseng, P. C. Celeste, A. H. Flood, Y. Liu, J. F. Stoddart, J. I. Zink, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 10029 – 10034; j) 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; k) T. D. Nguyen, K. C.-F. Leung, M. Liong, Y. Liu, J. F. Stoddart, J. I. Zink, *Adv. Funct. Mater.* **2007**, *17*, 2101 – 2110; l) M. Fujiwara, S. Terashima, Y. Endo, K. Shiokawa, H. Ohue, *Chem. Commun.* **2006**, 4635 – 4637; m) R. Liu, X. Zhao, T. Wu, P. Feng, *J. Am. Chem. Soc.* **2008**, *130*, 14418 – 14419.

[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] E. Climent, R. Martínez-Máñez, F. Sancenón, M. D. Marcos, J. Soto, A. Maquieira, P. Amorós, *Angew. Chem.* **2010**, *122*, 7439 – 7441; *Angew. Chem. Int. Ed.* **2010**, *49*, 7281 – 7283.

[6] 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) A. Bernardos, L. Mondragón, E. Aznar, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, J. M. Barat, E. Pérez-Payá, C. Guillem, P. Amorós, *ACS Nano* **2010**, *4*, 6353 – 6358.

[7] a) N. K. Mal, M. Fujiwara, Y. Tanaka, *Nature* **2003**, *421*, 350 – 353; b) N. K. Mal, M. Fujiwara, Y. Tanaka, T. Taguchi, M. Matsukata, *Chem. Mater.* **2003**, *15*, 3385 – 3394; c) Y. Zhu, M. Fujiwara, *Angew. Chem.* **2007**, *119*, 2291 – 2294; *Angew. Chem. Int. Ed.* **2007**, *46*, 2241 – 2244; d) N. G. Liu, Z. Chen, D. R. Dunphy, Y.-B. Jiang, R. A. Assink, C. J. Brinker, *Angew. Chem.* **2003**, *115*, 1773 – 1776; *Angew. Chem. Int. Ed.* **2003**, *42*, 1731 – 1734; e) 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 – 554; f) S. Angelos, E. Choi, F. Vögtle, L. De Cola, J. I. Zink, *J. Phys. Chem. C* **2007**, *111*, 6589 – 6592; g) J. Lu, E. Choi, F. Tamanoi, J. I. Zink, *Small* **2008**, *4*, 421 – 426; h) J. L. Vivero-Escoto, I. I. Slowing, C.-W. Wu, V. S.-Y. Lin, *J. Am. Chem. Soc.* **2009**, *131*, 3462 – 3463; i) E. Aznar, R. Casasús, B. García-Acosta, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, P. Amorós, *Adv. Mater.* **2007**, *19*, 2228 – 2231; j) E. Aznar, M. D. Marcos, R. Martínez-Máñez, F. Sancenón, J. Soto, P. Amorós, C. Guillem, *J. Am. Chem. Soc.* **2009**, *131*, 6833 – 6843.

[8] a) Q. Fu, G. V. R. Rao, L. K. Ista, Y. Wu, B. P. Andrzejewski, L. A. Sklar, T. L. Ward, G. P. López, *Adv. Mater.* **2003**, *15*, 1262 – 1266; b) Q. Fu, G. V. R. Rao, T. L. Ward, Y. Lu, G. P. López, *Langmuir* **2007**, *23*, 170 – 174; c) Z. Y. Zhou, S. M. Zhu, D. Zhang, *J. Mater. Chem.* **2007**, *17*, 2428 – 2433; d) S. Zhu, Z. Zhou, D. Zang, C. Jin, Z. Li, *Microporous Mesoporous Mater.* **2007**, *106*, 56 – 61; e) Y. Zhu, S. Kaskel, T. Ikoma, N. Haganata, *Microporous Mesoporous Mater.* **2009**, *123*, 107 – 112; f) Y.-Z. You, K. K. Kalebaila, S. L. Brock, D. Oupicky, *Chem. Mater.* **2008**, *20*, 3354 – 3359; g) C. Liu, J. Guo, W. Yang, J. Hu, C. Wang, S. Fu, *J. Mater. Chem.* **2009**, *19*, 4764 – 4770; h) 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.

[9] a) J. S. Beck, J. C. Vartuli, W. J. Roth, M. E. Leonowicz, C. T. Kresge, K. D. Schmitt, C. T.-W. Chu, D. H. Olson, E. W. Sheppard, S. B. McCullen, J. B. Higgins, J. L. Schlenker, *J. Am. Chem. Soc.* **1992**, *114*, 10834 – 10843; b) A. P. Wight, M. E. Davis, *Chem. Rev.* **2002**, *102*, 3589 – 3614; c) G. Kickelbick, *Angew. Chem.* **2004**, *116*, 3164 – 3166; *Angew. Chem. Int. Ed.* **2004**, *43*, 3102 – 3104; d) A. Stein, *Adv. Mater.* **2003**, *15*, 763 – 775.

