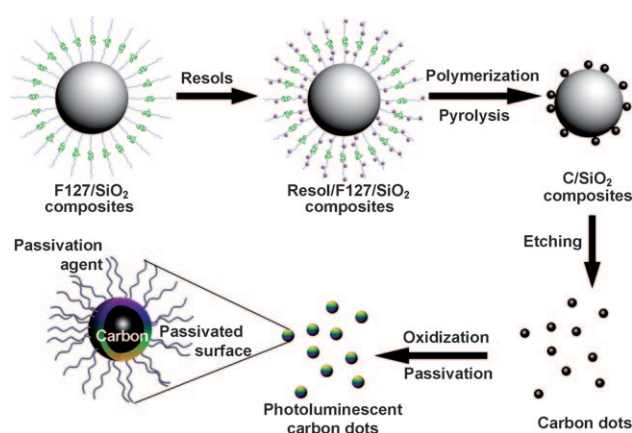


An Aqueous Route to Multicolor Photoluminescent Carbon Dots Using Silica Spheres as Carriers**

Ruili Liu,* Dongqing Wu, Shuhua Liu, Kaloian Koynov, Wolfgang Knoll, and Qin Li*

The emergence of photoluminescent carbon-based nanomaterials (CNMs) has presented exciting opportunities^[1] in the search for benign “nanolanters” that are highly desired in bioimaging, disease detection, and drug delivery. Compared to fluorescent semiconductor nanocrystals (quantum dots, QDs),^[2] photoluminescent CNMs are superior in chemical inertness and biocompatibility and potentially have low toxicity.^[1c] After the first report on the photoluminescence of carbon nanotubes,^[3] intense research has focused on the preparation of these versatile materials.^[4] Nanodiamonds, synthesized by highly energetic processes, such as detonation, shock-wave, and high-energy electron-beam methods, have also demonstrated stable photoluminescence.^[5] Most recently, it was discovered that nanosized carbon dots prepared by laser ablation of graphite and subsequent surface passivation can be excited by a broad range of wavelengths and give off bright photoluminescence.^[6] Photoluminescent CNMs also extend to carbon nanoparticles separated from candle soot, but these have a very low quantum yield of about 1%.^[7] Herein we report a novel and straightforward route towards nanosized (1.5–2.5 nm) carbon dots (CDs) with amorphous

structure by employing surfactant-modified silica spheres as carriers. In particular, our preparation method is wet-chemistry-based and requires no elaborate equipment, which presents a general and economic approach to produce photoluminescent CDs. Other important features are the CDs’ good biocompatibility and high photoluminescence efficiencies of 11–15% in a wide range of pH values from 5 to 9.



Scheme 1. Processing diagram for the synthesis of multicolor photoluminescent carbon dots.

The overall synthetic procedure is illustrated in Scheme 1. First, satellite-like polymer/F127/silica composites were prepared by an aqueous route using silica colloid spheres functionalized with amphiphilic triblock copolymer F127 ($\text{EO}_{106}\text{PO}_{70}\text{EO}_{106}$, $M_w = 12\,600$; EO = ethylene oxide, PO = propylene oxide) as carriers and resols (phenol/formaldehyde resins, $M_w < 500$) as carbon precursors. The subsequent high-temperature treatment and removal of silica carriers generated nanosized CDs. Acid treatment and simple surface passivation finally resulted in water-soluble, multicolor photoluminescent CDs. The key of our method is the employment of surfactant-modified silica nanospheres as carriers, which not only provide anchors for the polymerization of resols in solution but also prevent the aggregation of the nanosized carbon dots during pyrolysis.

Typically, colloidal SiO_2 suspension (5 g, 10 wt %, ca. 120 nm)^[8] was added to 10 mL aqueous solution containing 1.0 g F127. The mixture was stirred overnight at room temperature. After the removal of excessive surfactant by centrifugation (7000 rpm, 10 min), the surfactant-modified silica spheres were redispersed into aqueous NaOH (100 mL, pH 9). Meanwhile, freshly prepared resols (6 mL) in basic conditions^[9] were added as carbon precursors. The resols are

[*] Dr. R. Liu, Dr. D. Wu, Dr. S. Liu, Dr. K. Koynov, Prof. Dr. W. Knoll, Dr. Q. Li
Max-Planck-Institut für Polymerforschung
Ackermannweg 10, 55128 Mainz (Germany)
Fax: (+49) 6131-379-100
E-mail: liur@mpip-mainz.mpg.de
liqin@mpip-mainz.mpg.de

Dr. Q. Li
Department of Chemical Engineering,
Curtin University of Technology, Perth, WA 6845 (Australia)
E-mail: q.li@curtin.edu.au

Dr. S. Liu
Division of Bioengineering, Faculty of Engineering
National University of Singapore, Singapore 119260 (Singapore)
Prof. Dr. W. Knoll
Austrian Research Centers, Donau-City-Strasse 1, 1220 Vienna (Austria)

[**] Published on the occasion of the 25th anniversary of the Max Planck Institute for Polymer Research, Mainz.

We thank Dr. Y. Zhang for the AFM characterization, K. Kirchhoff for the TEM characterization, and Prof. N. Heckenberg for the helpful discussions. R.L. and S.L. acknowledge Alexander von Humboldt (AvH) Fellowships. S.L. also thanks Singapore Millennium Foundation (SMF) for financial support. Q.L. is grateful to Dr. E.-K. Sinner's Bio-lab at the Max Planck Institute for Polymer Research for hosting, the technical assistance from M. Droege, E. Conrad, and A. Arslan, and the partial support from Australian Research Council under DP0558727.



Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/ange.200900652>.

assumed to adsorb on the F127, forming the resol/F127/SiO₂ composites. The mixture was stirred at 66 °C for 48 h to allow for the polymerization of the resol precursors on the surfactant shells. After centrifugation (7000 rpm, 10 min) and drying at room temperature, khaki-colored polymer/F127/silica composites were obtained. The satellite-like morphology of the as-made composites can be clearly observed in the SEM and TEM images (Figure S1A–C in the Supporting Information), confirming that resols were polymerized on the surface of silica spheres. From the SEM and TEM images, the diameters of these polymer particles were estimated to be in the range of 10–40 nm.

In a parallel control experiment, direct mixing of unmodified SiO₂ colloid spheres and resols only resulted in separated layers of khaki-colored polymer nanoparticles (top) and white silica spheres (bottom) after centrifugation. This result indicates that resols prefer to polymerize in the solution rather than on the surface of unmodified silica spheres, because the latter cannot provide sufficient hydroxy groups to anchor resol adsorption through hydrogen bonds. Therefore, the modification of SiO₂ spheres with surfactant F127 is the prerequisite for preparing such satellite-like polymer/silica composites.

On the basis of the reaction phenomena, the following formation mechanism of the satellite-like polymer/F127/silica composites is proposed: When surfactant F127 is added to the SiO₂ colloids, micellization-like self-assembly^[10] occurs on the silica surface (Scheme 1). After the removal of excessive surfactant, F127/silica composites are re-dispersed into aqueous solution (pH 9), where the copolymer chains on the silica surface are fully extended by interacting with water, and core-shell silica/surfactant composites are formed (F127/SiO₂ composites in Scheme 1). Since the open surfactant shell makes it much easier for guest molecules to enter into the shell layer, the loading is enhanced compared to the unmodified silica sphere surface. Specifically, the hydrophilic domains, that is, the PEO blocks of F127, provide more anchors for resol precursors and have strong interactions with them through hydrogen bonds. Under continuous stirring at 66 °C in a weakly basic solution, polymerization takes place between the resols on the surface of silica spheres, leading to the formation of satellite-like polymer/F127/SiO₂ composites.

Subsequent calcination of these satellite-like polymer/F127/silica composites at 900 °C in Ar for 2 h led to corresponding carbon/silica composites. During this process, surfactant was completely removed by thermal decomposition (350–400 °C in Ar), while dehydrogenation and further polymerization of resols took place, accompanied by volume shrinkage.^[9,11] Generally, to reduce their high surface energy, nanoparticles with high surface-to-volume ratios tend to aggregate into large particles during high-temperature treatment. However, our synthesis strategy circumvents this problem by exploiting silica submicrometer particles as the carriers. To release the carbon dots, the silica spheres were removed by etching with 2 M NaOH solution at 40 °C for 48 h. The excessive NaOH was first neutralized by HNO₃ and then removed by dialyzing the supernatant against Milli-Q water through a dialysis membrane (MWCO 1000). However, owing to the increased structural density after high-temper-

ature treatment, it is difficult to completely etch off the silica spheres. Some remaining silica spheres with small nanoparticles on their surface can still be observed in the TEM image of the crude sample (Figure S1D in the Supporting Information), which appear to be the parent CDs/silica composite particles. The TEM image provides compelling evidence of how CDs are distributed on the silica spheres. As mentioned above, before calcination the polymer spheres attached on the silica particles were 10–40 nm in diameter. Therefore, it is curious to observe that after calcination and etching, the carbonized particles have diameters of only a few nanometers (Figure S1D in the Supporting Information). The mechanism of this drastic size reduction has remained elusive but is under further investigation. In the following procedure, upon high-speed centrifugation (10000 rpm, 30 min), both CDs/silica composites and any large carbon nanoparticles derived from CDs/silica composites were removed as precipitates, and a light yellow suspension containing crude CDs was obtained. No characteristic peaks of SiO₂ can be detected in the Fourier transform infrared (FTIR) spectroscopy (Figure S2a in the Supporting Information) of the resultant carbon dots, suggesting that silica was completely removed. The Raman spectrum (Figure S3 in the Supporting Information) reveals that the resultant crude carbon dots possess both sp² and sp³ hybrids, indicating an amorphous nature. This finding may be related to the carbon precursors, which are used to make phenolic resins (a kind of hard carbon source) and the low calcination temperature (900 °C), and it is in good agreement with our previous results.^[9,11] Elemental analysis shows that the crude CDs are composed of C 90.32 wt %, H 1.36 wt % and O (calculated) 8.34 wt %.

To introduce carboxy groups to the surface of CDs, the crude CD suspension was mixed with a quarter volume of concentrated nitric acid (end concentration 3 M), and the mixture was heated at reflux for 24 h.^[6] Similar to the procedure above, the excessive acid was first neutralized by Na₂CO₃ and then removed by dialyzing against Milli-Q water. The suspension appeared as a homogeneous light-yellow solution (Figure S4a in the Supporting Information). FTIR spectroscopy (Figure S2b in the Supporting Information) of oxidized CDs shows a sharp peak at 1737 cm⁻¹ assigned to the C=O stretching vibrations, thus indicating the presence of carbonyl groups. This result is consistent with increased oxygen content, as observed by the elemental analysis: C 78.17 wt %, H 5.08 wt %, and O (calculated) 16.75 wt %. Unlike the carbon nanoparticles separated from candle soot,^[7] the suspension of such oxidized CDs cannot emit luminescence under excitation at 365 nm (Figure S4c in the Supporting Information). Surface passivation is essential for these CDs to attain photoluminescence emission, similar to CDs prepared by laser ablation of graphite.^[6] A typical surface passivation process was carried out as follows: 50 mg diamine-terminated oligomeric poly(ethylene glycol), H₂NCH₂(CH₂CH₂O)_nCH₂CH₂CH₂NH₂ (*n*_{av} ≈ 35, PEG_{1500N}) was added to 20 mL oxidized carbon dots solution and then subjected to ultrasonication for 10 min to form a homogeneous suspension. The mixture was subsequently heated at 120 °C for 72 h for surface passivation. The optically transparent and photoluminescent CD suspension was purified by

dialyzing against Milli-Q water with a cellulose ester membrane bag ($M_w=3500$), during which excess PEG_{1500N} was removed. After filtering through 0.2 μm teflon, a clear, light yellow aqueous suspension containing PEG_{1500N}-passivated carbon dots was finally obtained (Figure S4b in the Supporting Information). Strong photoluminescence was observed when this suspension was irradiated by a 365 nm UV lamp (Figure S4d in Supporting Information). As shown by the FTIR spectra (Figure S2d in the Supporting Information), the broadened peak at 1737 cm^{-1} indicates the co-existence of $\nu_{\text{C=OOH}}$ and $\nu_{\text{C=ONR}}$ vibrations, suggesting that a fraction of the carbonyl groups has been converted into amide groups during the passivation process. The other characteristic peaks provide further evidence for the formation of amide groups: a small peak at 1604 cm^{-1} assigned to $\delta_{\text{CON-HR}}$ and a broad moderate peak at 3440 cm^{-1} assigned to $\nu_{\text{CON-HR}}$. Elemental analysis reveals that the composition of CDs passivated with PEG_{1500N} is C 64.65 wt %, H 7.67 wt %, N 1.13 wt %, and O (calculated) 26.55 wt %.

As shown in Figure 1, characterization by both high-resolution transmission electron microscopy (HRTEM) and atomic force microscopy (AFM) suggests that the size of the

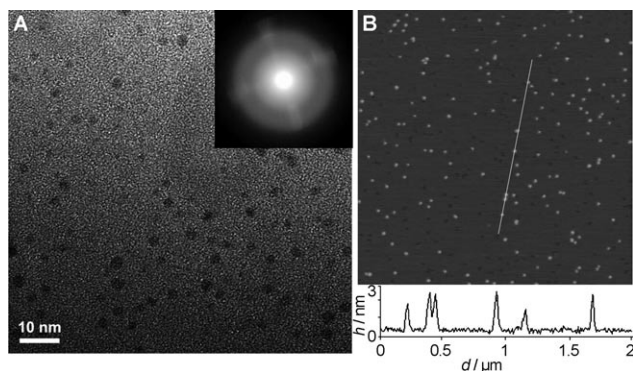


Figure 1. A) HRTEM image of carbon dots surface-passivated with PEG_{1500N}. The inset is the SAED pattern. B) AFM topography image of carbon dots on mica substrates with the height profile along the line in the image.

CDs is narrowly distributed with diameters in the range of 1.5–2.5 nm. The absence of discernible lattice structures on the HRTEM image (Figure 1A) indicates that the resultant CDs are amorphous, which is further confirmed by the diffuse ring pattern obtained by selected area electron diffraction (SAED; inset in Figure 1A). This finding is consistent with the Raman analysis. Dynamic light scattering (DLS) results (not shown) indicated that the average hydrodynamic diameter of PEG_{1500N} in methanol is less than 1 nm. The average hydrodynamic diameter of the carbon dots in water was determined by fluorescence correlation spectroscopy (FCS)^[12] to be 2.8 nm. This value is in agreement with the AFM and TEM characterizations, as the hydrodynamic diameter is usually slightly larger than the dry-state diameter owing to the PEG layer and the hydration effect. Moreover, the good single-component fit to the FCS autocorrelation curve (Figure S5 in the Supporting Information) confirms the narrow size distribution of the carbon dots.

The solution-like PEG_{1500N}-passivated CDs suspension shows a broad UV/Vis absorption (Figure 2) and exhibits strong blue luminescence under excitation at 365 nm (see

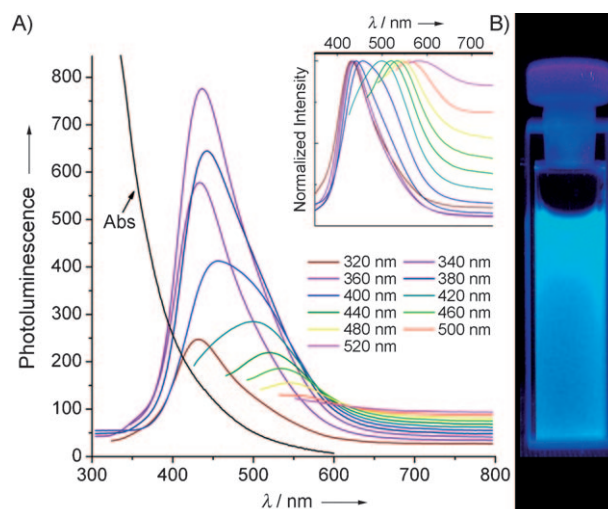


Figure 2. A) UV/Vis absorption (Abs) and photoluminescence emission spectra (recorded for progressively longer excitation wavelengths from 320 to 520 nm in 20 nm increments) of carbon dots surface-passivated with PEG_{1500N} in water. In the inset, the emission spectral intensities are normalized. B) Optical photograph obtained under excitation at 365 nm.

photograph in Figure 2 and Figure S4d in the Supporting Information). As shown in Figure 2, the emission spectra of these carbon dots are broad, ranging from 430 (violet) to 580 nm (yellow), with a dependence on the excitation wavelengths. It should be pointed out that neither the oxidized CDs nor the organic passivation agent (PEG_{1500N}) alone are obviously nonemissive in the UV/Vis range (Figures S4c,e and S6 in the Supporting Information). Therefore, the bright and colorful photoluminescence from the final carbon dots may be attributed to the presence of surface energy traps that become emissive upon stabilization as a result of the surface passivation.^[6] The requirement of surface passivation for photoluminescence is apparently shared by carbon dots^[6] and quantum dots,^[2a] for which a widely accepted mechanism for luminescence emission is the radiative recombination of excitations. The multicolor photoluminescence arises not only from CDs of different sizes in the sample but also from a distribution of different emissive trap sites.^[6] By selecting quinine sulfate as the standard and 360 nm as the excitation wavelength, the photoluminescent quantum yield of the PEG_{1500N}-passivated CDs was measured and calculated to be 14.7 % (Figure S7 in the Supporting Information). The value is comparable to that for low-toxicity InP–ZnS core-shell QDs (10–15 %)^[2c] and for laser-ablated graphite (4–10 %).^[6] Furthermore, the CDs are stable in a wide range of pH values (pH 5–9) with only a slight decrease in the photoluminescence quantum yields (11.0 and 12.1 % for pH 5 and 9, respectively) and no shift of the emission peak when excited at 360 nm (Figure S7 in the Supporting Information). This decrease of quantum yield could be related to the

change of the conditions surrounding the CDs' passivated surfaces, which may provide a hint as to the mechanism of CD photoluminescence.

Laser scanning confocal microscopy studies have shown that such PEG_{1500N}-capped surface-passivated CDs have very good biocompatibility as bioimaging agents. Figure 3 shows

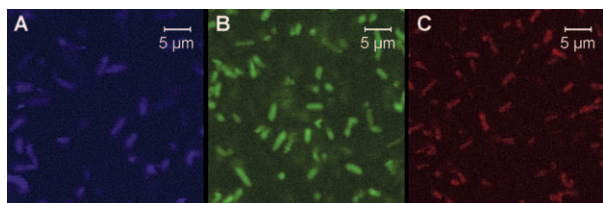


Figure 3. Confocal microscopy images of *E. coli* ATCC 25922 cells labeled with the carbon dots. A) $\lambda_{\text{EX}} = 458$ nm, detected with 475 nm long-pass filter; B) $\lambda_{\text{EX}} = 488$ nm, detected with 505 nm long-pass filter; C) $\lambda_{\text{EX}} = 514$ nm, detected with 530 nm long-pass filter. *E. coli* cells were grown in Invitrogen ImMedia™ Amp liquid medium at 37°C for 24 h and subsequently washed twice with phosphate-buffered saline (PBS; 1000 rpm, 10 min). The cell concentration was adjusted to approximately 10^8 cfu mL⁻¹ (optical density 0.52 at 600 nm). 200 μ L carbon dots suspension (1.1 μ M) was directly added into 5 mL of such a PBS cell suspension. The mixture was kept at 37°C, 155 rpm for 24 h before the images were recorded with a confocal laser scanning microscope (LSM510, Carl Zeiss, Germany).

confocal microscopy images of *E. coli* cells labeled with the CDs after a co-incubation of 24 h, where the *E. coli* cells appear to be completely covered by the CDs. The photoluminescence can be collected with a broad range of excitation wavelengths (e.g. 458, 488, and 514 nm, Figure 3). The CDs were also found to be easily internalized in murine P19 progenitor cells (Figure S8 in the Supporting Information). The versatility in surface modification offers an ideal platform for formulating a water-stable and targeted bioimaging tracer. Moreover, the laser scanning confocal microscopy analyses also indicated that the photostability of these CDs is remarkably high, with no blinking and low photobleaching.

In summary, we have developed a general and facile chemical synthesis method to fabricate multicolor photoluminescent CDs with amorphous structures. The CDs with diameter 1.5–2.5 nm were derived from satellite-like carbon/silica composites, which were prepared using surfactant-modified silica spheres as the carriers and resols as the carbon precursor. The surfactant, triblock copolymer F127, which served as a binder between silica spheres and resols, is found to be the deciding factor in the formation of satellite-like polymer/F127/silica composites. The quantum yield of the PEG_{1500N}-passivated CDs was characterized to be 14.7%, which is the highest value among the CDs reported to date. A significant advantage of this direct, bottom-up chemical synthetic route lies in its low cost and high flexibility. This method may be extended to the synthesis of photoluminescent CDs with different compositions through variation of the

carbon precursors, such as N-containing pyrrole, B- and F-doped polymers, and so forth. Such varied compositions could allow controlled adjustment of their emission wavelength range and assist us in understanding the intrinsic photoluminescence mechanisms for carbon nanomaterials. Furthermore, such versatile surface-passivated CDs have demonstrated very good biocompatibility, and they thus hold promise as potential bioimaging agents offering single-molecule resolution owing to their tiny size of 1.5–2.5 nm.

Received: February 3, 2009

Published online: April 22, 2009

Keywords: bioimaging · carbon · nanoparticles · photoluminescence

- [1] a) L. Cao, X. Wang, M. J. Meziani, F. S. Lu, H. F. Wang, P. J. G. Luo, Y. Lin, B. A. Harruff, L. M. Veca, D. Murray, S. Y. Xie, Y. P. Sun, *J. Am. Chem. Soc.* **2007**, *129*, 11318–11318; b) B. S. Harrison, A. Atala, *Biomaterials* **2007**, *28*, 344–353; c) K. Welscher, Z. Liu, D. Daranciang, H. Dai, *Nano Lett.* **2008**, *8*, 586–590; d) P. W. Barone, S. Baik, D. A. Heller, M. S. Strano, *Nat. Mater.* **2005**, *4*, 86–92.
- [2] a) A. P. Alivisatos, *Science* **1996**, *271*, 933–937; X. Michalet, F. F. Pinaud, L. A. Bentolila, J. M. Tsay, S. Doose, J. J. Li, G. Sundaresan, A. M. Wu, S. S. Gambhir, S. Weiss, *Science* **2005**, *307*, 538–544; b) U. Resch-Genger, M. Grabolle, S. Cavaliere-Jaricot, R. Nitschke, T. Nann, *Nat. Methods* **2008**, *5*, 763–775; c) X. H. Gao, Y. Y. Cui, R. M. Levenson, L. W. K. Chung, S. M. Nie, *Nat. Biotechnol.* **2004**, *22*, 969–976; d) A. M. Derfus, W. C. W. Chan, S. N. Bhatia, *Nano Lett.* **2004**, *4*, 11–18; e) D. J. Bharali, D. W. Lucey, H. Jayakumar, H. E. Pudavar, P. N. Prasad, *J. Am. Chem. Soc.* **2005**, *127*, 11364–11371.
- [3] J. E. Riggs, Z. X. Guo, D. L. Carroll, Y. P. Sun, *J. Am. Chem. Soc.* **2000**, *122*, 5879–5880.
- [4] a) S. Banerjee, S. S. Wong, *J. Am. Chem. Soc.* **2002**, *124*, 8940–8948; b) D. A. Tsybouski, J. D. R. Rocha, S. M. Bachilo, L. Cognet, R. B. Weisman, *Nano Lett.* **2007**, *7*, 3080–3085; c) L. J. Carlson, S. E. Maccagnano, M. Zheng, J. Silcox, T. D. Krauss, *Nano Lett.* **2007**, *7*, 3698–3703; d) X. Y. Xu, R. Ray, Y. L. Gu, H. J. Ploehn, L. Gearheart, K. Raker, W. A. Scrivens, *J. Am. Chem. Soc.* **2004**, *126*, 12736–12737.
- [5] a) S. J. Yu, M. W. Kang, H. C. Chang, K. M. Chen, Y. C. Yu, *J. Am. Chem. Soc.* **2005**, *127*, 17604–17605; b) A. Krueger, *Adv. Mater.* **2008**, *20*, 2445–2449.
- [6] Y. P. Sun, B. Zhou, Y. Lin, W. Wang, K. A. S. Fernando, P. Pathak, M. J. Meziani, B. A. Harruff, X. Wang, H. F. Wang, P. J. G. Luo, H. Yang, M. E. Kose, B. L. Chen, L. M. Veca, S. Y. Xie, *J. Am. Chem. Soc.* **2006**, *128*, 7756–7757.
- [7] H. P. Liu, T. Ye, C. D. Mao, *Angew. Chem.* **2007**, *119*, 6593–6595; *Angew. Chem. Int. Ed.* **2007**, *46*, 6473–6475.
- [8] W. Stöber, A. Fink, E. Bohn, *J. Colloid Interface Sci.* **1968**, *26*, 62–69.
- [9] F. Q. Zhang, Y. Meng, D. Gu, Y. Yan, C. Z. Yu, B. Tu, D. Y. Zhao, *J. Am. Chem. Soc.* **2005**, *127*, 13508–13509.
- [10] P. K. Sudeep, T. Emrick, *Polym. Rev.* **2007**, *47*, 155–163.
- [11] R. L. Liu, Y. F. Shi, Y. Wan, Y. Meng, F. Q. Zhang, D. Gu, Z. X. Chen, B. Tu, D. Y. Zhao, *J. Am. Chem. Soc.* **2006**, *128*, 11652–11662.
- [12] R. Rigler, E. Elson, *Fluorescence Correlation Spectroscopy: Theory and Applications*, Springer, Berlin, **2001**.