Synthetic Receptors

DOI: 10.1002/anie.201206514

Narrowly Dispersed Hydrophilic Molecularly Imprinted Polymer Nanoparticles for Efficient Molecular Recognition in Real Aqueous Samples Including River Water, Milk, and Bovine Serum**

*Yue Ma, Guoqing Pan, Ying Zhang, Xianzhi Guo, and Huiqi Zhang**

Molecular imprinting has proven to be a versatile approach to the preparation of synthetic receptors with tailor-made recognition sites.[1,2] The ultimate goal of molecular imprinting is to generate molecularly imprinted polymers (MIPs) with affinity and specificity approaching those of the biological receptors so that they can eventually replace such biological entities in real applications. However, previously developed MIPs that target small organic molecules are normally only compatible with organic solvents, and they mostly fail to show specific template binding in aqueous solutions (whereas peptide- or protein-imprinted polymers are intrinsically water-compatible),[3] which significantly limits their practical application in such areas as molecularly imprinted sorbent assays and biomimetic sensors.[4] Despite some progress made in the development of MIPs applicable for analyte detection in relatively simple aqueous media, such as pure water,[5] surfactant-containing water,[6] aqueous buffer solutions (mostly containing an organic solvent),[4b,7] and beer or solutions that mimic alcoholic beverages,[8] the design of MIPs directly capable of specifically recognizing the targeted small organic molecules in real biological samples remains a formidable challenge owing to the complex nature of the sample matrices.[4d,e,h]

Herein, we report the efficient synthesis of narrowly dispersed hydrophilic MIP nanoparticles with excellent specific molecular-recognition ability in real aqueous solutions, including river water and biological samples (both diluted and undiluted milk and bovine serum). Reversible addition–fragmentation chain-transfer (RAFT) precipitation polymerization (RAFTPP)[5a–c,g] mediated by hydrophilic macromolecular chain-transfer agents (macro-CTAs) provided for the first time narrowly dispersed highly cross-linked MIP (or polymer) nanoparticles with surface-grafted hydrophilic polymer brushes in a facile one-pot approach (Figure 1). The hydrophilic polymer brushes on the MIP nanoparticles not only significantly improved their surface hydrophilicity and led to their water compatibility,[5a–c,f,g] but they also acted as a protective layer to prevent proteins in the biological samples from accumulating on the nanoparticle surface and blocking the imprinting sites[9,10] and thus enabled the MIP nanoparticles to function properly in such complex matrices. Although studies on the use of MIPs in biological media for specific molecular recognition have been disclosed,[11] either diluted biological solutions (containing 40 vol% of a mixture of ethanol/water (1:1 v/v) and a phosphate buffer) were used[11a] or the targeted molecule was a highly water soluble 26 amino acid peptide (i.e., the bee toxin melittin).[11b] In this context, there have been many reports on the MIP-based solid-phase extraction of target analytes in real matrices;[12] however, the selectivity of the MIPs in this case is controlled by the choice of solvents in the extraction procedure. The direct use of MIPs in real matrices is more difficult, as the conditions used are mainly fixed by the nature of the sample.[4e] To our knowledge, we report herein the first successful example of the synthesis of MIP nanoparticles that can be used directly in undiluted biological samples for the efficient specific recognition of small organic molecules. This finding is a major breakthrough for molecular-imprinting technology, since it opens the door to the facile preparation of nanoscale MIPs (with good dispersion and outstanding performance in real aqueous samples) that are very attractive synthetic substitutes for biological receptors in bioanalytical applications and many other fields.

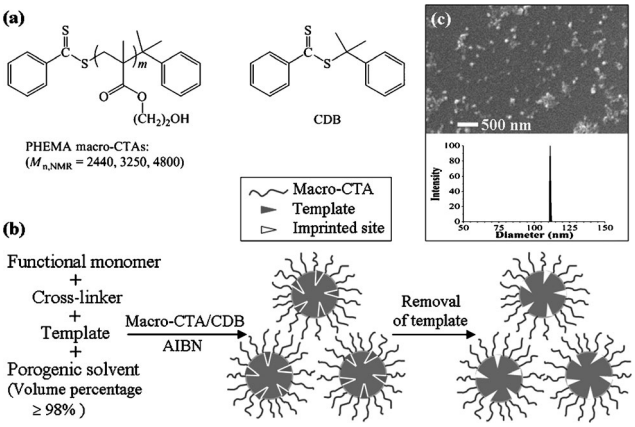

Figure 1. Preparation and characterization of MIP nanoparticles. a) Chemical structures of the RAFT agents used: hydrophilic macro-CTAs and CDB. b) Schematic protocol for the one-pot preparation by RAFTPP of MIP nanoparticles compatible with real aqueous solutions. c) Characterization of MIP nanoparticles ($M_{n,NMR}$ of PHEMA brushes: 4800) by SEM and DLS.

[*] Y. Ma, G. Pan, Y. Zhang, X. Guo, Prof. Dr. H. Zhang
Key Laboratory of Functional Polymer Materials
Ministry of Education, Department of Chemistry, Nankai University
Tianjin 300071 (P.R. China)
E-mail: zhanghuiqi@nankai.edu.cn

[**] We gratefully acknowledge financial support from the National Natural Science Foundation of China (20744003, 20774044, 21174067), the Natural Science Foundation of Tianjin (11JCYBJC01500), and the New Century Excellent Talents Program (Ministry of Education; NCET-07-0462).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201206514.

To demonstrate the general principle, we first chose a model noncovalent molecular imprinting system which used 2,4-dichlorophenoxyacetic acid (2,4-D; see Scheme S1 in the Supporting Information), 4-vinylpyridine (4-VP), ethylene glycol dimethacrylate (EGDMA), and a mixture of methanol and water (4:1 v/v) as the template, the functional monomer, the cross-linker, and the porogenic solvent, respectively. We carried out RAFTPP to prepare 2,4-D-imprinted polymers (2,4-D-MIPs) with azobisisobutyronitrile (AIBN) as the initiator in the presence of a chain-transfer agent (RAFT agent) and a large amount of the porogenic solvent ($\geq 98\,\%$ of the total reaction volume). Important features of our approach were the compatibility of all reactants with both RAFT polymerization and the molecular-imprinting processes, and the ability of 4-VP to form a hydrophobic interaction and an ionic bond with 2,4-D in polar solvents.[7a]

Three hydrophilic poly(2-hydroxyethyl methacrylate) (PHEMA) polymers with a dithioester end group and different molecular weights (M_n) were readily synthesized by RAFT polymerization and used as the macro-CTAs for RAFTPP (Figure 1a; see also Table S1 in the Supporting Information). A series of 2,4-D-MIPs and their control polymers (2,4-D-CPs) were readily obtained by RAFTPP mediated by a mixture of one macro-CTA and a normal RAFT agent (cumyl dithiobenzoate, CDB; see Table S2). In such polymerization systems, the macro-CTAs acted as both co-chain-transfer agents and steric stabilizers in the synthesis of the desired MIP and CP particles with surface-grafted hydrophilic PHEMA brushes.[13] Ungrafted 2,4-D-MIP and 2,4-D-CP were also prepared by RAFTPP by using only CDB as the RAFT agent and were used as controls for the grafted polymers in the following studies.

An SEM study revealed that the ungrafted 2,4-D-MIP and 2,4-D-CP were narrowly dispersed microspheres with number-average diameters (D_n) around 2 μm (see Figure S2 and Table S2 in the Supporting Information), whereas the grafted polymers were rather small nanoparticles (typically $D_n < 100$ nm in the dry state; Figure 1c; see also Figure S2). A further study by dynamic light scattering (DLS) showed that the grafted MIPs and CPs were narrow or monodisperse particles with hydrodynamic diameters (111–178 nm) in solution that were somewhat larger than the D_n values found by SEM (see Figure S3 and Table S2).[14] The presence of the characteristic peaks for the hydroxy O–H stretching band around 3540 cm^{-1} in the FTIR spectra of the grafted MIPs and CPs (Figure 2a), together with their significantly reduced static water contact angles in comparison with those of the ungrafted polymers (Figure 2b; see also Table S2) and their high dispersion stability in water (Figure 2c; see also Figure S5), was strong evidence for the successful grafting of hydrophilic polymer brushes. Furthermore, the rather similar grafting levels of hydrophilic polymer brushes on the grafted MIP and corresponding CP nanoparticles were also revealed by their FTIR spectra and rather close static water contact angles.

The equilibrium binding properties of the ungrafted and grafted 2,4-D-MIPs/2,4-D-CPs were first studied in an organic-solvent-rich medium (methanol/water (4:1 v/v)) and in pure water. Both the ungrafted and the grafted MIPs were found to bind more of the template than their corresponding CPs (see Figure S6a) and showed high selectivity towards the template (see Figure S13a and Table S3) in methanol/water (4:1 v/v). These results suggest the presence of specific binding sites in the MIPs. As expected, the specific template binding (i.e., the binding difference between the MIP and its CP)[14] of the ungrafted MIP almost completely disappeared in the pure aqueous solution owing to its high surface hydrophobicity (see Figure S6b).[7d] In sharp contrast, the grafted MIPs showed specific template binding in pure aqueous media rather close to that observed in methanol/water (4:1 v/v) because of their greatly improved surface hydrophilicity (see Figure S6b).[5a–c,f,g] Moreover, they also exhibited clear selectivity towards the template in pure water (see Figure S13b and Table S3).

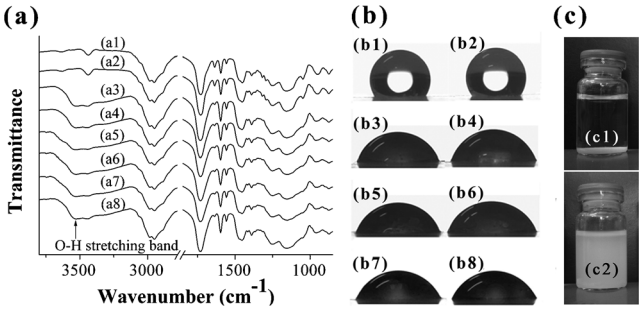

Figure 2. a) FTIR spectra of the ungrafted MIP (a1), the ungrafted CP (a2), and the grafted MIPs (a3, a5, a7) and CPs (a4, a6, a8) bearing PHEMA brushes with $M_{n,NMR} = 2440$ (a3/a4), 3250 (a5/a6), and 4800 (a7/a8). b) Profiles of a water drop on films of the ungrafted MIP (b1), the ungrafted CP (b2), and the grafted MIPs (b3, b5, b7) and CPs (b4, b6, b8) bearing PHEMA brushes with $M_{n,NMR} = 2440$ (b3/b4), 3250 (b5/b6), and 4800 (b7/b8). c) Photographs showing the dispersion of the ungrafted MIP (c1, after standing for 1.5 h) and the grafted MIP bearing PHEMA brushes with $M_{n,NMR} = 4800$ (c2, after standing for 5 days) in water at 25 °C (MIP template: 2,4-D).

We then performed the equilibrium binding experiments in real aqueous solutions. In the first step, the template-binding experiments were carried out in river water and some diluted biological samples, including milk/water (1:1 v/v) and bovine serum/water (1:1 v/v). The batch adsorption study revealed that the grafted MIP bearing PHEMA brushes with $M_{n,NMR} = 4800$ bound more of the template than its corresponding CP in all aqueous media studied (Figure 3a), and its specific template binding was rather close to that observed in both pure water and methanol/water (4:1 v/v; Figure 3b). Moreover, the grafted MIP also showed high selectivity towards the template in river water, milk/water, and bovine serum/water (see Figure S14d and Table S3). These results clearly demonstrated that the grafted MIP nanoparticles bearing PHEMA brushes with $M_{n,NMR} = 4800$ had superior molecular-recognition ability in these aqueous solutions.

The effect of the molecular weight of the hydrophilic macro-CTA used (i.e., the chain length of the grafted polymer brushes) on the equilibrium binding properties of the resulting MIPs and CPs in river water and diluted biological samples was also investigated. It can be seen clearly that, in contrast to the MIP nanoparticles grafted with relatively long PHEMA brushes ($M_{n,NMR} = 4800$), those bearing shorter PHEMA brushes ($M_{n,NMR} = 2440$ and 3250) could only

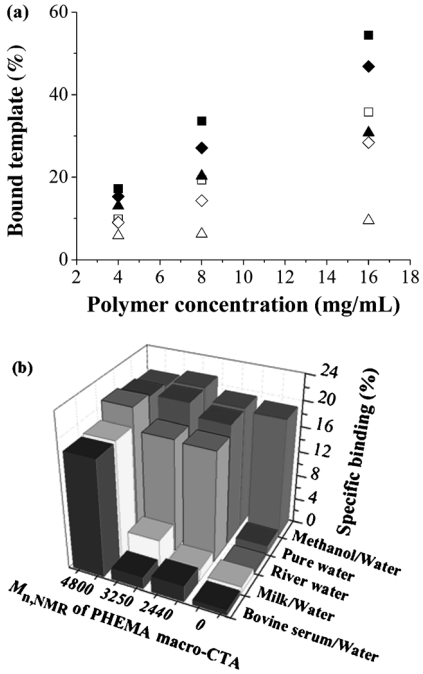

Figure 3. a) Equilibrium binding of 2,4-D (0.02 mM) to different amounts of the grafted MIP (filled symbols) and CP (open symbols) bearing PHEMA brushes ($M_{n,NMR}=4800$) in river water (triangle), milk/water (1:1 v/v; square), and bovine serum/water (1:1 v/v; diamond) at 25 °C. b) Specific template binding to the ungrafted MIP (i.e., M_n of the macro-CTA is 0) and grafted MIPs bearing PHEMA brushes with $M_{n,NMR}=2440$, 3250, and 4800 in solutions of 2,4-D (0.02 mM) in different media at 25 °C (MIP template: 2,4-D; polymer concentration: 16 mg mL^{-1}).

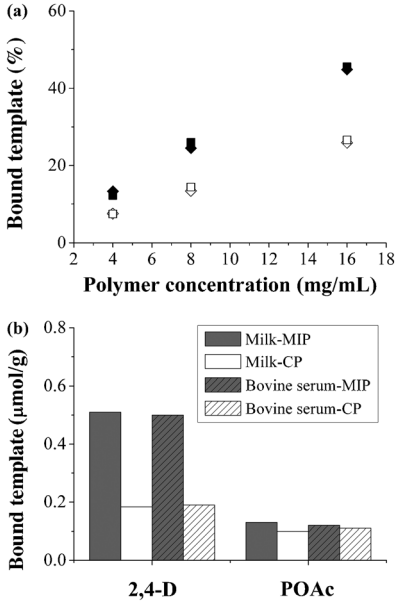

Figure 4. a) Equilibrium binding of 2,4-D (0.02 mM) to different amounts of the grafted MIP (filled symbols) and CP (open symbols) bearing PHEMA brushes with $M_{n,NMR}=4800$ in undiluted milk (square) and bovine serum (diamond) at 25 °C. b) Selective binding of the grafted MIP and CP bearing PHEMA brushes with $M_{n,NMR}=4800$ towards a mixture of 2,4-D (0.02 mM) and POAc (phenoxyacetic acid, see Scheme S1) (0.02 mM) in undiluted milk and bovine serum at 25 °C (MIP template: 2,4-D, polymer concentration: 12 mg mL^{-1}).

specifically recognize the template in river water, whereas their specific template binding (Figure 3b; see also Figure S7b,c) and selectivity towards the template (see Figure S14b,c and Table S3) almost disappeared in the diluted milk and bovine serum. These results suggest that the chain length of the hydrophilic polymer brushes played a decisive role in the compatibility of the grafted MIP nanoparticles with biological samples, and that only those polymer brushes with a high enough molecular weight could act as an efficient hydrophilic protective shield for the MIP nanoparticles to prevent the accumulation of proteins on their surface and enable them to function properly.[10b,c] As expected, the ungrafted MIP hardly showed any specific template binding in river water or diluted biological solutions (Figure 3b; see also Figures S7a and S14a and Table S3).

Next, equilibrium binding experiments were performed with the best candidate in the above study (i.e., the grafted 2,4-D-MIP bearing PHEMA brushes with $M_{n,NMR}=4800$) in undiluted milk and bovine serum. The grafted MIP could clearly bind more of the template than the corresponding CP (Figure 4a). More importantly, its specific template binding proved to be rather similar to that observed in methanol/water, pure water, river water, and diluted biological media. These results, together with the high selectivity of this grafted MIP towards the template in milk and bovine serum (Figure 4b; see also Table S3), clearly confirmed its excellent specific molecular-recognition ability in the real biological samples.

Finally, narrowly dispersed hydrophilic propranolol-imprinted polymer nanoparticles were also prepared by RAFTPP mediated by a PHEMA macro-CTA ($M_{n,NMR}=4800$; see Table S2 and Figures S2–S5). The resulting nanoparticles also showed excellent compatibility with river water, undiluted milk, and bovine serum (see Figures S9 and S16 and Table S4). This result, together with the total incompatibility of ungrafted propranolol-MIP microspheres prepared by RAFTPP (see Figures S9 and S16 and Table S4) as well as conventional 2,4-D and propranolol-imprinted polymers (see Figure S11) with such real aqueous solutions, definitively verified the general applicability and high versatility of our strategy in the solution of this long-standing problem in molecular imprinting with the efficient specific recognition of small organic molecules in complex aqueous media.

In conclusion, we have demonstrated for the first time the efficient one-pot synthesis of narrowly dispersed hydrophilic MIP nanoparticles that are fully compatible with a series of real aqueous solutions (including river water, diluted and undiluted milk, and bovine serum) by RAFTPP mediated by hydrophilic macro-CTAs. The chain length of the polymer brushes on the MIP nanoparticles had a significant influence on the compatibility of the nanoparticles with biological samples: only sufficiently long polymer brushes could act as an efficient protective layer to prevent the accumulation of proteins on the nanoparticle surface and thus enable the nanoparticles to function properly in such a complex milieu. Furthermore, we were able to confirm the general applicability of our strategy. In view of the versatility of RAFT polymerization in the field of molecular imprinting,[5a–c,g,15] the ready preparation of these narrowly dispersed MIP nano-

particles, and their excellent dispersion and binding properties in biological samples, we believe that such synthetic MIP nanoparticles are highly promising alternatives to biological receptors with great potential in many analytical applications (e.g., for environmental, food, and clinical analyses) and other areas.

Received: August 13, 2012
Revised: November 27, 2012
Published online: December 18, 2012

Keywords: biological samples · molecular recognition · polymers · precipitation polymerization · synthetic receptors

[1] a) K. Haupt, K. Mosbach, *Chem. Rev.* **2000**, *100*, 2495 – 2504; b) B. Sellergren, *Angew. Chem.* **2000**, *112*, 1071 – 1078; *Angew. Chem. Int. Ed.* **2000**, *39*, 1031 – 1037; c) G. Wulff, *Chem. Rev.* **2002**, *102*, 1 – 28; d) J. Z. Hilt, M. E. Byrne, *Adv. Drug Delivery Rev.* **2004**, *56*, 1599 – 1620; e) C. Alexander, H. S. Andersson, L. I. Andersson, R. J. Ansell, N. Kirsch, I. A. Nicholls, J. O'Mahony, M. J. Whitcombe, *J. Mol. Recognit.* **2006**, *19*, 106 – 180; f) H. Zhang, L. Ye, K. Mosbach, *J. Mol. Recognit.* **2006**, *19*, 248 – 259; g) L. Ye, K. Mosbach, *Chem. Mater.* **2008**, *20*, 859 – 868; h) B. Sellergren, *Nat. Chem.* **2010**, *2*, 7 – 8; i) K. Haupt, *Nat. Mater.* **2010**, *9*, 612 – 614; j) Y. Hoshino, K. J. Shea, *J. Mater. Chem.* **2011**, *21*, 3517 – 3521; k) M. J. Whitcombe, I. Chianella, L. Larcombe, S. A. Piletsky, J. Noble, R. Porter, A. Horgan, *Chem. Soc. Rev.* **2011**, *40*, 1547 – 1571; l) K. Haupt, A. V. Linares, M. Bompart, B. Tse Sum Bui, *Top. Curr. Chem.* **2012**, *325*, 1 – 28.

[2] a) *Molecularly Imprinted Polymers*: *Man-Made Mimics of Antibodies and Their Applications in Analytical Chemistry* (*Techniques and Instrumentation in Analytical Chemistry*; Ed.: B. Sellergren), Elsevier Science, Amsterdam, **2001**; b) *Molecular Imprinting*: *From Fundamentals to Applications* (Eds.: M. Komiyama, T. Takeuchi, T. Mukawa, H. Asanuma), Wiley-VCH, Weinheim, **2003**; c) *Molecularly Imprinted Materials*: *Science and Technology* (Eds.: M. Yan, O. Ramström), Marcel Dekker, New York, **2005**; d) *Molecular Imprinting of Polymers* (Eds.: S. Piletsky, A. Turner), Landes Bioscience, Georgetown, **2006**.

[3] a) H. Shi, W. Tsai, M. D. Garrison, S. Ferrari, B. D. Ratner, *Nature* **1999**, *398*, 593 – 597; b) B. R. Hart, K. J. Shea, *J. Am. Chem. Soc.* **2001**, *123*, 2072 – 2073; c) H. Nishino, C. S. Huang, K. J. Shea, *Angew. Chem.* **2006**, *118*, 2452 – 2456; *Angew. Chem. Int. Ed.* **2006**, *45*, 2392 – 2396; d) A. Cutivet, C. Schembri, J. Kovensky, K. Haupt, *J. Am. Chem. Soc.* **2009**, *131*, 14699 – 14702; e) A. Nematollahzadeh, W. Sun, C. S. A. Aureliano, D. Lütkemeyer, J. Stute, M. J. Abdekhodaie, A. Shojaei, B. Sellergren, *Angew. Chem.* **2011**, *123*, 515 – 518; *Angew. Chem. Int. Ed.* **2011**, *50*, 495 – 498; f) M. J. Whitcombe, *Nat. Chem.* **2011**, *3*, 657 – 658; g) X. Shen, T. Zhou, L. Ye, *Chem. Commun.* **2012**, *48*, 8198 – 8200.

[4] a) G. Vlatakis, L. I. Andersson, R. Müller, K. Mosbach, *Nature* **1993**, *361*, 645 – 647; b) L. I. Andersson, R. Müller, G. Vlatakis, K. Mosbach, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 4788 – 4792; c) K. Haupt, *Chem. Commun.* **2003**, 171 – 178; d) N. Lavignac, C. J. Allender, K. R. Brain, *Anal. Chim. Acta* **2004**, *510*, 139 – 145; e) V. Pichon, F. Chapuis-Hugon, *Anal. Chim. Acta* **2008**, *622*, 48 – 61; f) Y. Ge, A. P. F. Turner, *Chem. Eur. J.* **2009**, *15*, 8100 – 8107; g) T. Fodey, P. Leonard, J. O'Mahony, R. O'Kennedy, M. Danaher, *TrAC Trends Anal. Chem.* **2011**, *30*, 254 – 269; h) M. C. Moreno-Bondi, M. E. Benito-Peña, J. L. Urraca, G. Orellana, *Top. Curr. Chem.* **2012**, *325*, 111 – 164.

[5] a) G. Pan, Y. Zhang, X. Guo, C. Li, H. Zhang, *Biosens. Bioelectron.* **2010**, *26*, 976 – 982; b) G. Pan, Y. Ma, Y. Zhang, X. Guo, C. Li, H. Zhang, *Soft Matter* **2011**, *7*, 8428 – 8439; c) G. Pan, Y. Zhang, Y. Ma, C. Li, H. Zhang, *Angew. Chem.* **2011**, *123*, 11935 – 11938; *Angew. Chem. Int. Ed.* **2011**, *50*, 11731 – 11734; d) X. Shen, L. Ye, *Macromolecules* **2011**, *44*, 5631 – 5637; e) X. Shen, L. Ye, *Chem. Commun.* **2011**, *47*, 10359 – 10361; f) L. Fang, S. Chen, X. Guo, Y. Zhang, H. Zhang, *Langmuir* **2012**, *28*, 9767 – 9777; g) Y. Ma, Y. Zhang, M. Zhao, X. Guo, H. Zhang, *Chem. Commun.* **2012**, *48*, 6217 – 6219.

[6] Z. Meng, W. Chen, A. Mulchandani, *Environ. Sci. Technol.* **2005**, *39*, 8958 – 8962.

[7] a) K. Haupt, A. Dzgoev, K. Mosbach, *Anal. Chem.* **1998**, *70*, 628 – 631; b) C. Lübke, M. Lübke, M. J. Whitcombe, E. N. Vulfson, *Macromolecules* **2000**, *33*, 5098 – 5105; c) J. G. Karlsson, L. I. Andersson, I. A. Nicholls, *Anal. Chim. Acta* **2001**, *435*, 57 – 64; d) B. Dirion, Z. Cobb, E. Schillinger, L. I. Andersson, B. Sellergren, *J. Am. Chem. Soc.* **2003**, *125*, 15101 – 15109; e) Y. Watabe, K. Hosoya, N. Tanaka, T. Kubo, T. Kondo, M. Morita, *J. Chromatogr. A* **2005**, *1073*, 363 – 370; f) C. E. Hunt, P. Pasetto, R. J. Ansell, K. Haupt, *Chem. Commun.* **2006**, 1754 – 1756; g) J. L. Urraca, A. J. Hall, M. C. Moreno-Bondi, B. Sellergren, *Angew. Chem.* **2006**, *118*, 5282 – 5285; *Angew. Chem. Int. Ed.* **2006**, *45*, 5158 – 5161; h) J. Oxelbark, C. Legido-Quigley, C. S. A. Aureliano, M. M. Titirici, E. Schillinger, B. Sellergren, J. Courtois, K. Irgum, L. Dambies, P. A. G. Cormack, D. C. Sherrington, E. D. Lorenzi, *J. Chromatogr. A* **2007**, *1160*, 215 – 226; i) K. Yang, M. M. Berg, C. Zhao, L. Ye, *Macromolecules* **2009**, *42*, 8739 – 8746.

[8] a) P. Manesiotis, A. J. Hall, J. Courtois, K. Irgum, B. Sellergren, *Angew. Chem.* **2005**, *117*, 3970 – 3974; *Angew. Chem. Int. Ed.* **2005**, *44*, 3902 – 3906; b) P. Manesiotis, C. Borrelli, C. S. A. Aureliano, C. Svensson, B. Sellergren, *J. Mater. Chem.* **2009**, *19*, 6185 – 6193.

[9] a) T. Cedervall, I. Lynch, M. Foy, T. Berggård, S. C. Donnelly, G. Cagney, S. Linse, K. A. Dawson, *Angew. Chem.* **2007**, *119*, 5856 – 5858; *Angew. Chem. Int. Ed.* **2007**, *46*, 5754 – 5756; b) Y. Hoshino, H. Koide, K. Furuya, W. W. Haberaecker III, S.-H. Lee, T. Kodama, H. Kanazawa, N. Oku, K. J. Shea, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 33 – 38.

[10] a) N. B. Holland, Y. Qiu, M. Ruegsegger, R. E. Marchant, *Nature* **1998**, *392*, 799 – 801; b) H. Ma, D. Li, X. Shen, B. Zhao, A. Chilkoti, *Langmuir* **2006**, *22*, 3751 – 3756; c) R. Barbey, L. Lavanant, D. Paripovic, N. Schüwer, C. Sugnaux, S. Tugulu, H.-A. Klok, *Chem. Rev.* **2009**, *109*, 5437 – 5527.

[11] a) H. Bengtsson, U. Roos, L. I. Andersson, *Anal. Commun.* **1997**, *34*, 233 – 235; b) Y. Hoshino, H. Koide, T. Urakami, H. Kanazawa, T. Kodama, N. Oku, K. J. Shea, *J. Am. Chem. Soc.* **2010**, *132*, 6644 – 6645.

[12] a) B. Sellergren, *Anal. Chem.* **1994**, *66*, 1578 – 1582; b) E. Caro, R. M. Marcé, F. Borrull, P. A. G. Cormack, D. C. Sherrington, *TrAC Trends Anal. Chem.* **2006**, *25*, 143 – 154; c) B. Tse Sum Bui, K. Haupt, *Anal. Bioanal. Chem.* **2010**, *398*, 2481 – 2492; d) E. Turiel, A. Martín-Esteban, *Anal. Chim. Acta* **2010**, *668*, 87 – 99.

[13] a) Z. An, Q. Shi, W. Tang, C. K. Tsung, C. J. Hawker, G. D. Stucky, *J. Am. Chem. Soc.* **2007**, *129*, 14493 – 14499; b) Y. Li, S. P. Armes, *Angew. Chem.* **2010**, *122*, 4136 – 4140; *Angew. Chem. Int. Ed.* **2010**, *49*, 4042 – 4046; c) W. Shen, Y. Chang, G. Liu, H. Wang, A. Cao, Z. An, *Macromolecules* **2011**, *44*, 2524 – 2530.

[14] K. Yoshimatsu, K. Reimhult, A. Krozer, K. Mosbach, K. Sode, L. Ye, *Anal. Chim. Acta* **2007**, *584*, 112 – 121.

[15] a) M. M. Titirici, B. Sellergren, *Chem. Mater.* **2006**, *18*, 1773 – 1779; b) C. H. Lu, W. H. Zhou, B. Han, H. H. Yang, X. Chen, X. R. Wang, *Anal. Chem.* **2007**, *79*, 5457 – 5461; c) A. D. Vaughan, J. B. Zhang, M. E. Byrne, *AIChE J.* **2010**, *56*, 268 – 279.

