Efficient strategy to increase the surface functionalization of core–shell superparamagnetic nanoparticles using dendron grafting†

Karine Heuzé,*[a] Daniel Rosario-Amorin,[a] Sylvain Nlate,[a] Manuel Gaboyard,[b] Anthony Bouter[cd] and Rodolphe Clérac[d]

Received (in Montpellier, France) 5th November 2007, Accepted 21st January 2008
First published as an Advance Article on the web 4th February 2008
DOI: 10.1039/b717027k

Core–shell γ-Fe_2O_3/polymer 300 nm superparamagnetic nanoparticles, grafted by fluorescent dendrons using a convergent approach, showed an increase in their surface functionalization compared to grafting using a linear analogue.

In recent years, superparamagnetic iron oxide nanoparticles[1] have shown great potential in many applications related to biotechnology and nanomaterials, such as biomedical applications (MRI, efficient enzyme or protein immobilization as an ELISA test, magnetically controlled transport of drugs)[2] and more recently as supported catalysts.[3] Most functionalized superparamagnetic nanoparticles (MNPs) are synthesized directly at the oxide surface (Fe_3O_4 or Fe_2O_3), but the key to their recent development has been the tremendous progress made in the surface chemistry of these nanomaterials, along with surface functionality needs and biocompatibility purposes for biotechnology applications. Two coating processes of superparamagnetic iron oxide nanoparticles have mainly been reported: (i) with a polymer shell or (ii) with a silica shell.[2–5] It is worth noting that new strategies have been explored, such as the direct functionalization of MNPs through phosphonic acid bridges[3i] for the immobilization of catalysts and the synthesis of nanoparticle heterodimers.[6]

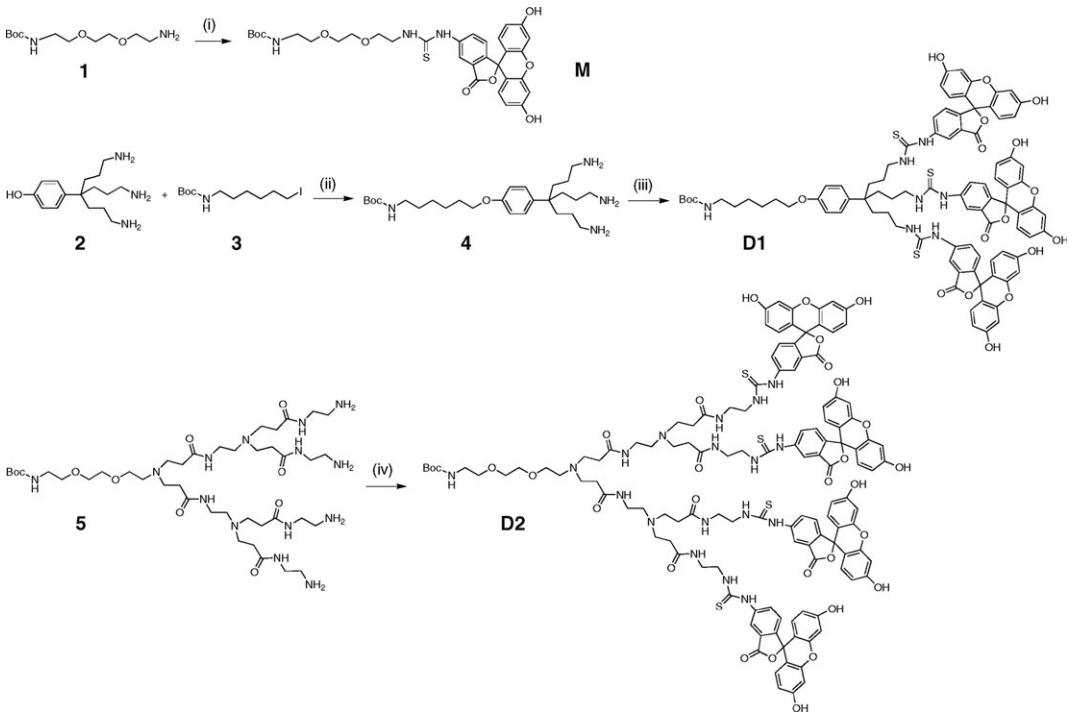
Dendrimers[7] are a class of macromolecules developed in the 1990s with a highly branched three-dimensional architecture. They have been used in many fields of chemistry (medical applications, nanoscience, catalysis) because of their intriguing properties. They are synthesized in an iterative sequence of reaction steps, in which each additional iteration leads to a higher generation material. The immobilization of dendrimers or dendritic structures on insoluble organic or inorganic supports (polymer, resin, silica surfaces) has been studied for a decade and offers a wide range of applications in various areas of chemistry, life and materials science.[8] However, only a few papers have been published in the field of dendrimers grafted or immobilized onto MNPs. Some of them report the synthesis of MNPs stabilized by dendritic architectures,[9] and most deal with either protein immobilization[10] or catalysis.[11] In the case of dendron-functionalized core–shell MNPs, the general synthetic approach reported is a divergent route, in which the dendron is built step by step on the MNPs' surface. In this Letter, we describe the synthesis of dendrons functionalized by a fluorescent tag and their grafting onto core–shell MNPs by a convergent approach.[12] In contrast to the divergent grafting approach, this method uses well-defined dendrons, since the dendritic parts are synthesized and characterized before their grafting on the MNPs' surface.[13] We show that this way of functionalization of core–shell MNPs leads to a high percentage of grafting, as demonstrated by the use of dendrons bearing fluorescent units on the MNPs' surface. As expected, the resulting dendron-grafted MNPs possess a larger number of fluorescent sites than those grafted with their linear analogue.

In order to demonstrate the widespread use of this grafting method, two dendritic structures have been studied. The **D1** dendron's backbone is mainly formed of hydrophobic components (alkyl chain and aromatic ring) and the **D2** backbone is mainly composed of hydrophilic components (polyether chain and polyamido amine chains) (Scheme 1). Indeed, the polarity of the dendron is often of great importance to the specific applications of the final material.

Monomer **M** and two dendrons, **D1** and **D2**, bearing a fluorescent tag (fluorescein), have been synthesized (Scheme 1) and grafted onto core–shell superparamagnetic nanoparticles. On the one hand, **D1** has been synthesized from the coupling of triaminophenol **2**[14] with the Boc-protected 6-iodo-1-aminohexane **3**, followed by the coupling between FITC (fluorescein isothiocyanate) and amino groups of the dendron. On the other hand, **D2** has been prepared according to the general method of Tomalia and co-workers[15] for the propagation of PAMAM (poly(amidoamine)) dendrimer generations. The first step is a Michael-type addition of the free amino group of a mono Boc-protected 2,2′-(ethylenedioxy)diethylamine **1** to methyl acrylate, forming the amino propionate ester. Then, amidation of the ester groups with ethylenediamine forms the first generation of the amino dendron. A repetition of these two steps (Michael-type addition followed by amidation) produces the four amino group-terminated

[a] *Institut des Sciences Moléculaires, Université Bordeaux 1, UMR CNRS No 5255, 351 cours de la Liberation, 33405 Talence cedex, France. E-mail: k.heuze@ism.u-bordeaux1.fr; Fax: +33 556 40 00 69 94; Tel: +33 556 40 00 62 74*
[b] *Ademtech, SA Parc scientifique Unitec, 4 allée du Doyen Georges Brus, 33600 Pessac, France*
[c] *ENITAB, IECB - Université Bordeaux 1, UMR CNRS No 5248 CBMN, 2 rue Robert Escarpit, 33607 Pessac, France*
[d] *Université Bordeaux 1, CNRS, Centre de Recherche Paul Pascal (CRPP), UPR CNRS No 8641, 115 avenue du Dr Schweitzer, 33600 Pessac, France*

† Electronic supplementary information (ESI) available: Characterization details, confocal and optical microscopy analyses of the sample described in Table 1, entry 9, and magnetization curves for **D2**-grafted MNPs. See DOI: 10.1039/b717027k



Scheme 1 Synthesis of **M**, **D1** and **D2**: (i) FITC, MeOH, 50 °C, 20 h; (ii) KOH, DMF, −20 °C to RT, 2.5 h; (iii) FITC, MeOH, 45 °C, 24 h; (iv) FITC, MeOH, 50 °C, 20 h.

dendron **5**. The final step is the coupling between FITC and the amino groups of the dendron **5**, leading to **D2**.

Next, the covalent grafting of **M**, **D1** or **D2** onto core–shell γ-Fe_2O_3 was investigated. Core–shell γ-Fe_2O_3/polymer 300 nm superparamagnetic nanoparticles (Carboxyl-Adembeads 300 nm, Ademtech, France, 300 mmol COOH g^{-1} of particles) were used as the MNP starting material.[16] These particles are usually used for *in vitro* diagnostics (particularly in the fields of immunology and virology).

They are well defined particles (dispersity below 20%), highly magnetic materials (70% iron oxide), and show a controlled polymeric surface (functionalized with carboxylic acid groups).

The general grafting method used has been optimized from typical procedures,[2] involving the functionalization of these core–shell γ-Fe_2O_3 particles with biological molecules. Our grafting procedure is based on amide bond formation between an amine group and a carboxylic acid group. In accordance with both typical grafting procedures for such superparamagnetic nanoparticles and the solubility of dendrons **D1** and **D2**, two solvent conditions (DMF or aqueous) were investigated for the grafting experiments. For both procedures, the first step is *in situ*-deprotection of the amino group by TFA (trifluoroacetic acid). Next, the grafting method has been optimized by adjustment of the dendron loading (Table 1 and Table 2). In an aqueous medium, all the nanoparticles remained in a colloidal state (except Table 1, entry 9), presuming a generally good integrity of the nanoparticles' polymer shell during the grafting procedure.‡ It is noteworthy that at the highest loading of **D2** (Table 1, entry 9), clear aggregation of the nanoparticles is observed by confocal and analytical microscopy. In a DMF medium (Table 2), particles seem to be degraded, as was confirmed further by TEM and flow cytometry analysis. This result confirmed that the better grafting procedure occurred in the aqueous medium. Fluorescence measurements revealed that the grafting rate in the aqueous medium was about 10%, based on the particles polymer shell COOH loading (300 mmol COOH g^{-1} of particles).§ These results have not been corrected from the quenching phenomenon induced by the local environment of the chromophores, so the grafting rate has been particularly underestimated for dendritic species (especially in the case of **D2**).

Table 1 Grafting conditions of **M**, **D1** and **D2** onto Carboxyl-Adembeads 300 nm nanoparticles in water

Entry	Dendron or monomer	Quantity mmol mg^{-1} particles
1	**M**	0.5
2	**M**	1
3	**M**	2
4	**D1**	0.5
5	**D1**	1
6	**D1**	2
7	**D2**	0.5
8	**D2**	1
9	**D2**	2

TEM microscopy of both **D1**- and **D2**-grafted MNPs reveals that the particle shape and size remained unchanged, since the size distribution analysis of the final material was comparable with the size distribution of the starting nanoparticles (240–360 nm), at least in the case of grafting experiments in the aqueous medium (Fig. 1, left). On the other hand, it is

‡ Colloidal state was observed by confocal and optical microscopy for each sample. An example of sample in a colloidal state is given in Fig. 2.

§ See experimental section for details.

Table 2 Grafting conditions of **D1** and **D2** onto Carboxyl-Adembeads 300 nm nanoparticles in DMF

Entry	Dendron	Quantity mmol mg^{-1} particles
1	**D1**	0.094
2	**D1**	0.47
5	**D2**	0.15
6	**D2**	0.75
7	**D2**	11.5
8	**D2**	3.75

noteworthy that a slight degradation of the polymer shell is observed in the TEM images of nanoparticles grafted in the organic medium (Fig. 1, right), confirming that DMF is not an appropriate solvent in the grafting process of these particles.

Confocal microscopy analysis (Fig. 2) of **D1**- and **D2**-grafted nanoparticles shows a strong fluorescence emission at a fluorescein wavelength. In the case of **D1**-grafted nanoparticles, a comparison of the images from the optical and fluorescence modes indicates that all the nanoparticles located in the focal plane are fluorescent, highlighting the homogeneous grafting at the surface of the nanoparticles. Flow cytometry measurements (Fig. 3) also show a sharp shape to each signal, at least for the grafting in the aqueous medium (Fig. 3B). Moreover, a comparison of these histograms reveals that the more the molecule is loaded during the grafting experiment, the stronger the recorded fluorescence signal. This result indicates a high percentage of grafting for a high molecule loading, especially for monomer molecules. In the case of grafting in the organic medium (Fig. 3A), the shape of the signal is slightly broader for higher dendron loadings, indicating a possible adsorption phenomenon of dendrons on the nanoparticles' surface. A comparison of the fluorescence signal recorded during flow cytometry (Fig. 3C) of **M**-, **D1**- and **D2**-grafted MNPs (Table 1, entries 2, 5 and 8, respectively) shows a significant dendritic effect. In fact, **M**-modified MNPs bear less fluorescent units (11.7 RFU) (RFU = relative fluorescence unit) than **D1**- (31.8 RFU) and **D2**- (28.6 RFU) modified MNPs. Again, these results have not been corrected for the quenching phenomenon, so the **D2**-modified MNPs' RFU value must be an underestimate. Anyhow, this result confirms the crucial role of dendron molecules in increasing the functionalization ability of such grafted materials.

The magnetization curves of **D1** and **D2** dendron-grafted MNPs have been recorded between 1.8 K and 300 K (Fig. 4 and Fig. S2†). Both samples and the original nanoparticles have virtually identical magnetic properties, in accordance with superparamagnetic behaviour. Below 150 K, a hysteresis effect appears on the *M vs. H* data, and has been followed as a function of temperature (inset Fig. 4). The thermal variation of the coercive field (which reaches 150 Oe at 1.8 K) is strictly the same, confirming that the MNPs remain intact and that the size of the magnetic core of the particle is quasi-identical. This result is of crucial importance, since many applications of MNPs require integrity of their magnetic properties, even after surface chemical modification.

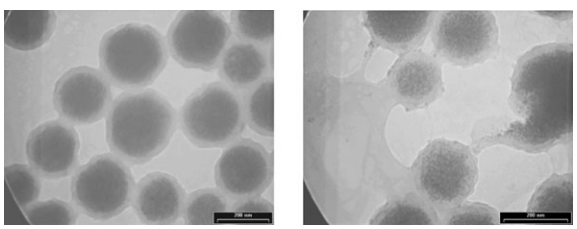


Fig. 1 TEM images of grafted γ-Fe_2O_3 Carboxyl-Adembeads 300 nm nanoparticles. Grafting conditions: Left **D2**: 0.15 mmol mg^{-1} of particles in water. Right **D1**: 0.1 mmol mg^{-1} of particles in DMF.

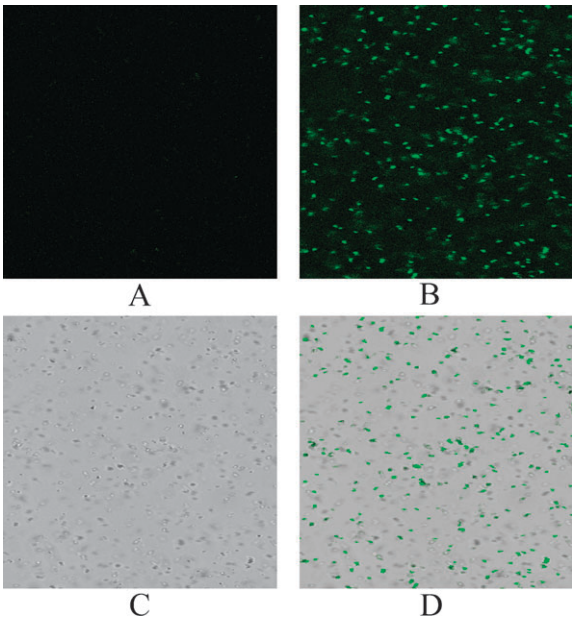


Fig. 2 Confocal microscopy and optical microscopy images (48 × 48 mm). A: Confocal microscopy image of γ-Fe_2O_3 Carboxyl-Adembeads 300 nm. B: Confocal microscopy image of γ-Fe_2O_3 Carboxyl-Adembeads 300 nm grafted with **D1**. C: Optical microscopy image of γ-Fe_2O_3 Carboxyl-Adembeads grafted with **D1**. D: Comparison of B and C images. Grafting conditions are described in Table 1, entry 4.

In summary, we have prepared core–shell superparamagnetic nanoparticles functionalized by fluorescein-modified dendrons. The fluorescence measurements of these nanoparticles confirm the ability of dendronized molecules to increase the surface functionalization of such MNPs, in contrast to their linear analogue. This alternative way of functionalization (convergent approach) of core–shell MNPs by well-defined dendritic molecules allows the tunable functionalization of the MNPs' surface. Also, the rate of functionalization increases with the generation of the dendron. We believe that this convergent approach for the grafting of MNPs would be a convenient way to better investigate dendritic effects,[17] which may be of great interest in the fields of biochemistry or catalysis.

Financial Support from the Centre National de la Recherche Scientifique (CNRS), the Université Bordeaux 1, the region Aquitaine and the Agence Nationale de la Recherche (ANR-05-JCJC-0245-01) is gratefully acknowledged. We thank Odile Babot at University Bordeaux 1, ISM for TGA measurements.

Experimental

Reagents

All reagents and solvents purchased were of analytical grade and used as received without further purification.

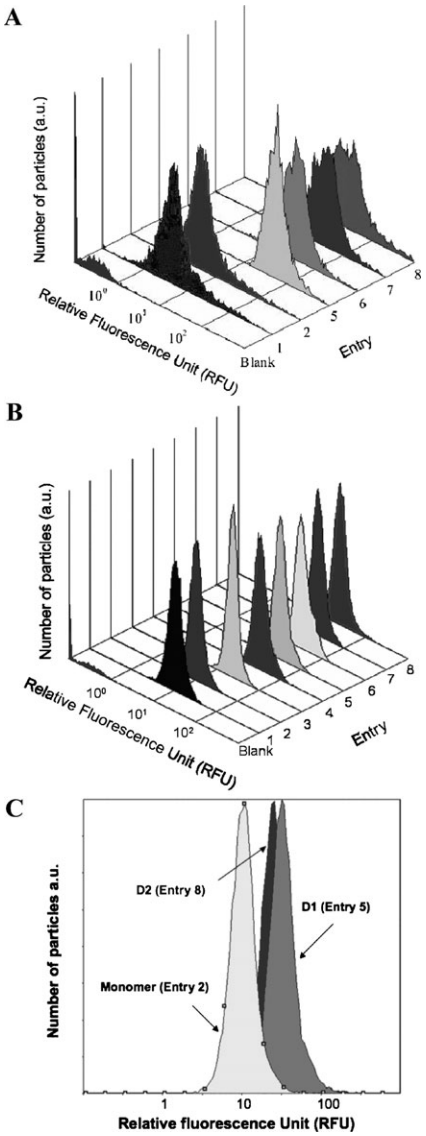


Fig. 3 Flow cytometry histograms of **M**, **D1** and **D2** grafted onto Carboxyl-Adembeads 300 nm nanoparticles. A: In DMF (see entries in Table 2). B: In water (see entries in Table 1). C: Comparison of **M**, **D1** and **D2** (see Table 1, entries 2, 5 and 8, respectively).

Synthesis of M

Mono Boc-protected 2,2′-(ethylenedioxy)diethylamine **1** (77 mg, 0.31 mmol) and FITC (120 mg, 0.31 mmol) were dissolved in MeOH (5 mL), and the mixture was heated at 50 °C for 20 h. The solvent was removed and the solid washed with CH_2Cl_2 and pentane. 131 mg (66% yield) of **M** was isolated as an orange solid. ^{1}H NMR (δ, d_6-DMSO): 8.28 (s, 1H, C*H*), 7.71 (d, 1H, C*H*), 7.15 (d, 1H, C*H*), 6.55 (m, 6H, C*H*), 3.66 (m, 2H, CH_2NHCS), 3.53 (m, 4H, CH_2O), 3.37 (m, 4H, CH_2O), 3.06 (q, 2H, CH_2NHBoc) and 1.35 (s, 9H, CH_3). ^{13}C NMR (δ, d_6-DMSO): 180.5 (*C*=S), 168.5 (*C*O–O), 178.7 (*C*ONH), 155.9 (*C*=O$_{Boc}$), 160.3 (*C*O–O), 155.5 (CO$_{Boc}$), 160.3, 152.1, 141.3, 129.0, 127.3, 124.3, 116.9, 113.0, 109.9, 102.2 (C_q + *C*H), 77.5 (C_{qBoc}), 69.4 (*C*H$_2$O), 43.6 (*C*H$_2$NHCS), 39.4 (*C*H$_2$NHBoc) and 28.1 (CH$_{3Boc}$). MS (CI): *m*/*z* calc. 636.7, found 636.3 (100) [M−H]$^+$.

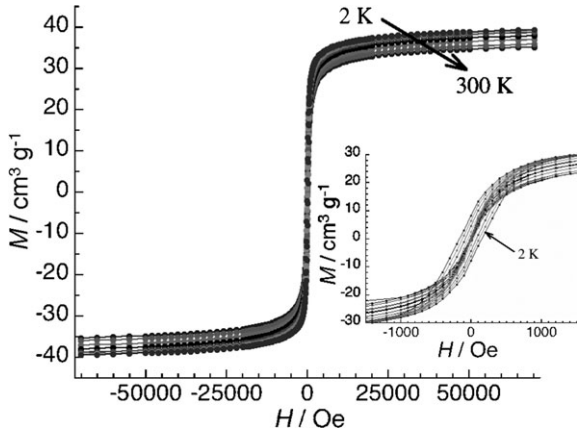


Fig. 4 Magnetization *vs.* applied magnetic field curves of **D1** dendron grafted γ-Fe_2O_3 Carboxyl-Adembeads 300 nm nanoparticles (grafting conditions are described in Table 1, entry 4). Inset: expanded view of the main figure (*M vs. H*) in the low field domain between −1500 and 1500 Oe, highlighting the hysteresis effect observed below 150 K.

Synthesis of D1

(a) *First step*. Coupling of triaminophenol dendron **2** with mono Boc-protected 6-iodo-1-aminohexane **3**: Triaminophenol dendron **2**[14] (1.99 g, 7.12 mmol) was dissolved in dry DMF and cooled to −20 °C. KOH (0.8 g, 14.3 mmol) was then added to the solution, and the mixture stirred for 15 min. A solution of mono Boc-protected 6-iodo-1-aminohexane **3** in DMF (6 mL) was added to the reaction mixture, and the temperature was increased slowly to RT (over 2.5 h). Water was added and the final product extracted with CH_2Cl_2. The organic layer was washed with water, dried over Na_2SO_4, and evaporated under reduce pressure to yield **4** as a colourless oil (2.38 g, 70%). ^{1}H NMR (δ, $CDCl_3$): 7.17 (d, 2H, CH_{ar}), 6.79 (d, 2H, CH_{ar}), 4.54 (br, 1H, N*H*), 3.91 (t, 2H, CH_2O), 3.10 (q, 2H, CH_2NH), 2.60 (t, 6H, CH_2NH$_2$), 1.75 (q, 2H, CH_2CH$_2$O), 1.59 (m, 6H, CH_2), 1.55–1.43 (m, CH_2CH$_2$N), 1.42 (s, 9H, CH_3), 1.42–1.20 (m, 4H, CH_2CH_2) and 1.17 (m, 6H, C_qCH_2). ^{13}C NMR (δ, $CDCl_3$): 156.85 (*C*O$_{ar}$), 156.1 (*C*=O), 139.1 ($C_{q(ar)}$), 127.4 (*C*H$_{ar}$), 114,0 (*C*H$_{ar}$), 79.1 ($C_{q(t\text{-}Bu)}$), 67.7 (*C*H$_2$O), 43.0 (*C*H$_2$NH$_2$), 42.1 ($C_{ar}C_q$), 40.1 (*C*H$_2$NH), 34.8 (*C*H$_2$CH$_2$NH$_2$), 30.1 (*C*H$_2$CH$_2$O), 29.4 (*C*H$_2$CH$_2$NH), 28.5 (*C*H$_3$), 28.0 (C_qCH$_2$), 26.7 (*C*H$_2$) and 25.9 (*C*H$_2$).

(b) *Second step*: A mixture of **4** (310 mg, 0.648 mmol) and FITC (756 mg, 1.94 mmol) was dissolved in MeOH (8 mL) and stirred at 45 °C for 24 h. The solvent was evaporated and the resulting precipitate washed with CH_2Cl_2 and MeOH to give **D1** as an orange powder (630 mg, 59% yield). ^{1}H NMR (δ, d_6-DMSO): 10.1 (br, 6H, O*H*), 8.16 (m, 3H, CH_{ar}), 7.52 (m, 3H, CH_{ar}), 7.17 (d, 3H, CH_{ar}), 6.84–6.56 (m, 18H, CH_{ar}) 3.85 (m, 2H, CH_2O), 3.54 (m, 6H, CH_2NHC=S), 2.99 (m, 2H, CH_2NH), 1.75 (m, 6H, CH_2–C_q), 1.62 (m, 2H, CH_2–CH$_2$O) and 1.41 (m, 21H, CH$_{3Boc}$ + CH_2–CH$_2$). Elemental analysis for $C_{90}H_{83}N_7O_{18}S_3$·11H_2O: calc. C, 58.59; H, 5.74; N, 5.31; S, 5.21; found C, 58.77; H, 5.57; N, 5.92; S, 5.48%. The presence of water molecules in the solid was confirmed by TGA measurements.

Synthesis of D2

We followed the general propagation procedure of dendrimers pioneered by Tomalia and co-workers.[15] A Michael-type

addition of methyl acrylate and mono Boc-protected 2,2′-(ethylenedioxy)diethylamine produced the amino propionate ester, followed by amidation of the resulting ester groups with ethylenediamine. A repetition of the two steps (Michael addition and amidation) afforded dendron **5**, which bears four terminal amine groups. Next, **5** (200 mg, 0.21 mmol) and FITC (334 mg, 0.86 mmol) were dissolved in MeOH (5 mL), and the mixture heated at 50 °C for 20 h. The red precipitate was washed with toluene and CH_2Cl_2, and dried under vacuum. We obtained 425 mg of **D2** (80% yield). ^{1}H NMR (δ, d_6-DMSO): 8.27 (m, 4H, CH_{ar}), 7.75 (m, 4H, CH_{ar}), 7.17 (d, 4H, CH_{ar}), 6.64–6.55 (m, 24H, CH_{ar}), 3.47–3.36 (m, 8H, CH_2O), 3.16 (m, 14H, CH_2NHC═O), 2.64 (m, 16H, CH_2N), 2.19 (m, 12H, CH_2CO) and 1.36 (s, 9H, CH_{3Boc}). Elemental analysis for $C_{125}H_{128}N_{18}O_{30}S_4$·10 H_2O: calc. C, 56.21; H, 5.59; N, 9.44; found C, 56.63; H, 5.59; N, 10.81%. The presence of water molecules in the solid was confirmed by TGA measurements.

General procedure for the grafting of dendrons D1 and D2 or M onto core–shell MNPs

A mixture of Carboxyl-Adembeads 300 nm (100 μL, solid content 1%) and a monomer or dendron solution of DMSO (20–160 μL, 0.5–2 mmol), EDC (*N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide; 80 μL, 6 mg mL^{-1}), NHS (*N*-hydroxysuccinimide; 80 μL, 12 mg mL^{-1}) and MES (morpholino ethyl sulfonic acid; pH = 6; to adjust the reaction mixture to 1 mL) was formed. Reaction mixture flasks were then shaken in rotators for 15 h. Next, particles were isolated with a magnet and washed several times with NaOH (10 mM) and dispersed in a Triton X405 solution. The procedure in the organic medium was the same as in the aqueous medium, except for the coupling reagent; CHMC (1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-*para*-toluene sulfonate) being used instead of EDC in this case.

Fluorescence analyses to determine the grafting rate of M, D1 and D2

Standard solutions of **M**, **D1** and **D2** in water containing free nanoparticles were prepared and measured (fluorescence intensity *vs.* N_{mole} mg^{-1} particles). Solutions of **M**, **D1** and **D2** grafted onto MNPs (see grafting conditions in Table 1, entries 2, 5 and 8, respectively) were analyzed. Grafting rates were calculated based on the particles' polymer shell COOH loading (300 mmol COOH g^{-1} of particles) and were found to be 8.4, 11.4 and 9.1% for **M**, **D1** and **D2** grafting, respectively.

References

1 U. Jeong, U. Teng, Y. Wang and Y. Xia, *Adv. Mater*., 2007, **19**, 33.
2 (*a*) A. K. Gupta and M. Gupta, *Biomaterials*, 2005, **26**, 3995; (*b*) J. Gao, L. Li, P.-L. Ho, G. C. Mak, H. Gu and B. Xu, *Adv. Mater*., 2006, **18**, 3145; (*c*) F. Hu, L. Wie, Z. Zhou, Y. Ran, Z. Li and M. Gao, *Adv. Mater*., 2006, **18**, 2553; (*d*) L. M. Rossi, A. D. Quach and Z. Rosenzweig, *Anal. Bioanal. Chem*., 2004, **380**, 606; (*e*) Q. A. Pankhurst, J. Connolly and S. K. Jones, *J. Phys. D: Appl. Phys*., 2003, **36**, R167; (*f*) P. Tartaj, M. del Puerto Morales, S. Ventemillias-Verdaguer, T. González-Carreño and C. J. Serna, *J. Phys. D: Appl. Phys*., 2003, **36**, R182; (*g*) C. C. Berry and A. S. G. Curtis, *J. Phys. D: Appl. Phys*., 2003, **36**, R198.
3 (*a*) S. Ding, Y. Xing, M. Radosz and Y. Shen, *Macromolecules*, 2006, **39**, 6399; (*b*) M. Kawamura and K. Sato, *Chem. Commun*., 2006, 4718; (*c*) N. T. S. Phan and C. W. Jones, *J. Mol. Catal. A: Chem*., 2006, **253**, 123; (*d*) Y. Zheng, P. D. Stevens and Y. Gao, *J. Org. Chem*., 2006, **71**, 537; (*e*) D. K. Yi, S. S. Lee and J. Y. Ying, *Chem. Mater*., 2006, **18**, 2459; (*f*) Z. Wang, B. Shen, Z. Aihua and N. He, *Chem. Eng. J*., 2005, **113**, 27; (*g*) P. D. Stevens, G. Li, J. Fan, M. Yen and Y. Gao, *Chem. Commun*., 2005, 4435; (*h*) P. D. Stevens, J. Fan, H. M. R. Gardimalla, M. Yen and Y. Gao, *Org. Lett*., 2005, **7**, 2085; (*i*) A. Hu, G. T. Yee and W. Lin, *J. Am. Chem. Soc*., 2005, **127**, 12486; (*j*) T.-J. Yoon, J. I. Kim and J.-K. Lee, *Inorg. Chim. Acta*, 2003, **345**, 228.
4 (*a*) S. Yu and G. M. Chow, *J. Mater. Chem*., 2004, **14**, 2781; (*b*) L. E. Euliss, S. G. Grancharov, S. O'Brien, T. J. Deming, G. Stucky, C. B. Murray and G. A. Held, *Nano Lett*., 2003, **3**, 1489; (*c*) C. R. Vestal and Z. J. Zhang, *J. Am. Chem. Soc*., 2002, **124**, 14312.
5 (*a*) T.-J. Yoon, J. S. Kim, B. G. Kim, K. N. Yu, M.-H. Cho and J.-K. Lee, *Angew. Chem., Int. Ed*., 2005, **44**, 1068; (*b*) D. K. Yi, S. T. Selvan, S. S. Lee, G. C. Papaefthymiou, D. Kundaliya and J. Y. Ying, *J. Am. Chem. Soc*., 2005, **127**, 4990; (*c*) A. P. Philipse, M. P. B. van Bruggen and C. Pathmamanoharan, *Langmuir*, 1994, **10**, 92.
6 H. Gu, Z. Yang, J. Gao, C. K. Chang and B. Xu, *J. Am. Chem. Soc*., 2005, **127**, 34.
7 (*a*) R. A. Kleij and P. W. N. M. van Leeuwen, *Eur. Pat*. 0456317, 1991; (*b*) P. W. N. M. van Leeuwen, *Chem. Abs*., 1992, **116**, 129870; (*c*) H. Brunner, J. Fürst and J. Ziegler, *J. Organomet. Chem*., 1993, **454**, 87; (*d*) For a review, see: H. Brunner, *J. Organomet. Chem*., 1995, **500**, 39; (*e*) A. W. Knappen, J. C. de Wilde, P. W. N. M. van Leeuwen, P. Wijkens, D. Grove and G. van Koten, *Nature*, 1994, **372**, 659; (*f*) A. W. Bosman, H. M. Janssen and E. W. Meijer, *Chem. Rev*., 1999, **99**, 1665; (*g*) R. Kreiter, A. W. Kleij, R. J. M. Klein Gebbink and G. van Koten, *Top. Curr. Chem*., 2001, **217**, 163; (*h*) J. J. Lee and W. T. Ford, *J. Am. Chem. Soc*., 1994, **116**, 3753; (*i*) G. R. Newkome, C. N. Moorefield and F. Vögtle, *Dendrimers and Dendrons: Concepts Syntheses and Applications*, Wiley-VCH, Weinheim, 2002.
8 (*a*) R. J. M. Klein Gebbink, C. A. Kruithof, G. P. M. van Klink and G. van Koten, *Rev. Mol. Biotechnol*., 2002, **90**, 183; (*b*) D. C. Tully and J.-M. J. Fréchet, *Chem. Commun*., 2001, 1229; (*c*) A. Dahan and M. Portnoy, *J. Polym. Sci., Part A: Polym. Chem*., 2005, **43**, 235; (*d*) G. R. Newkome, C. D. Weis and B. J. Childs, *Des. Monomers Polym*., 1998, **1**, 3; (*e*) S.-M. Lu and H. Alper, *J. Am. Chem. Soc*., 2006, **42**, 14776; (*f*) C. S. Love, I. Ashworth, C. Brennan, V. Chechik and D. K. Smith, *J. Colloid Interface Sci*., 2006, **302**, 178; (*g*) B. T. Mathews, A. E. Beezer, M. J. Snowden, M. J. Hardy and J. C. Mitchell, *New J. Chem*., 2001, **25**, 807.
9 (*a*) M. Kim, Y. Chen, Y. Liu and X. Peng, *Adv. Mater*., 2005, **17**, 1429; (*b*) E. Strable, J. W. M. Bulte, B. Moskowitz, K. Vivekanandan, M. Allen and T. Douglas, *Chem. Mater*., 2001, **13**, 2201; (*c*) B. L. Frankamp, A. K. Boal, M. T. Tuominen and V. M. Rotello, *J. Am. Chem. Soc*., 2005, **127**, 9731.
10 (*a*) B.-F. Pan, F. Gao and L.-M. Ao, *J. Magn. Magn. Mater*., 2005, **293**, 252; (*b*) B.-F. Pan, F. Gao and H.-C. Gu, *J. Colloid Interface Sci*., 2005, **284**, 1; (*c*) F. Gao, B.-F. Pan, W.-M. Zheng, L.-M. Ao and H.-C. Gu, *J. Magn. Magn. Mater*., 2005, **293**, 48.
11 R. Abu-Reziq, H. Alper, D. Wang and M. L. Post, *J. Am. Chem. Soc*., 2006, **128**, 5279.
12 C. Duanmu, I. Saha, Y. Zheng, B. M. Goodson and Y. Gao, *Chem. Mater*., 2006, **18**, 5973.
13 N. Tsubokawa, H. Ichioka, T. Satoh, S. Hayashi and K. Fujiki, *React. Funct. Polym*., 1998, **37**, 75.
14 S. Nlate, unpublished results.
15 (*a*) D. A. Tomalia, H. Baker, J. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, J. Ryder and P. Smith, *Polym. J*., 1985, **17**, 117; (*b*) D. A. Tomalia, H. Baker, J. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, J. Ryder and P. Smith, *Macromolecules*, 1986, **19**, 2466; (*c*) D. A. Tomalia, M. Hall and D. Hedstrand, *Macromolecules*, 1987, **20**, 1164.
16 (*a*) P. S. Doyle, J. Bibette, A. Bancaud and J. L. Viovy, *Science*, 2002, **295**, 2237; (*b*) Z. Bílková, A. Castagna, G. Zanusso, A. Farinazzo, S. Monaco, E. Damoc, M. Przybylski, M. Benes, J. Lenfeld, J.-L. Viovy and P. G. Richetti, *Proteomics*, 2005, **5**(3), 639; (*c*) N. Minc, C. Fütterer, K. D. Dorfman, A. Bancaud, C. Gosse, C. Goubault and J.-L. Viovy, *Anal. Chem*., 2004, **76**, 3770.
17 B. Helms and J.-M. J. Fréchet, *Adv. Synth. Catal*., 2006, **348**, 1125.