Surface Grafting of Functional Polymers to Macroporous Poly(trimethylolpropane trimethacrylate)

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Cross-linking polymerization of a trifunctional methacrylate monomer, trimethylolpropane trimethacrylate (TRIM), under controlled conditions yields macroporous polymers bearing surface-accessible unpolymerized methacrylate residues. These residues have been utilized for copolymerization with different functional monomers to obtain composite polymer matrices with surface coatings of functional polymer chains. Poly(TRIM) modified with methacrylamide, *N,N*-dimethylacrylamide, vinylazlactone, and copper(II) dimethacrylate exhibit useful functional properties that depend on the type of functional monomer used yet retain the desirable physical properties of the original poly(TRIM) matrix. The metal-complexing polymer made by grafting copper(II) dimethacrylate to poly(TRIM) exhibits better accessibility of the metal-coordinating sites compared to its bulk-polymerized counterpart. Similarly, the surface-hydrophilized matrices made by grafting methacrylamide and *N,N*-dimethylacrylamide show good water absorbency yet exhibit better matrix stability to environmental change compared to corresponding bulk-copolymerized materials. The physicochemical characteristics of these functional polymer matrices were evaluated by ^{13}C NMR, X-ray photoelectron spectroscopy, IR spectroscopy, and scanning electron microscopy. Poly(TRIM) particles surface-modified by template polymerization of a copper(II)–[*N*-(4-vinylbenzyl)imino]diacetic acid (**6**):template complex and ethylene glycol dimethacrylate exhibit selectivity similar to bulk-polymerized templated polymers in rebinding the bisimidazole template.

Introduction

This laboratory's interest in functional polymers stems from efforts to develop robust materials capable of selective substrate recognition and binding, important for applications ranging from separations to sensors. As part of this effort, we have been examining metal coordination as a basis for recognition of organic substrates in templated, or molecularly imprinted, polymers.[1] Synthesis of these templated polymers involves preorganization of a metal-coordinating template with a metal-containing monomer and subsequent polymerization of this monomer–template assembly with excess cross-linking agent. Complexation of metal-chelating monomers with the template during polymerization directs the positioning of metal ions in the resulting polymer matrices, while a high degree of cross-linking stabilizes the functional group arrangement to create specific recognition sites.[1b,c] Although the macroporous templated polymers can rebind low molecular weight substrates reversibly and with reasonable efficiency, the same is not true for large substrates, such as proteins, whose mobility within the polymer network is highly restricted. Furthermore, the templated polymers may not exhibit sufficient mechanical stability for the desired application. One approach to alleviating these problems is to make the binding sites accessible to the surface of the matrix by graft copolymerizing the monomer–template assemblies to a solid matrix possessing desirable physicomechanical properties.[1a,2]

The effectiveness of functional polymers in a range of settings from separations to solid-phase chemical synthesis has generated interest in developing new generations of such materials with improved thermomechanical stability yet high accessibility of functional groups.[3–6] Introduction of functional groups by grafting onto a polymer surface can yield materials possessing the desired bulk properties as well as the desired functional characteristics. An example is surface-functionalized porous silica for adsorptive separations.[7,8]

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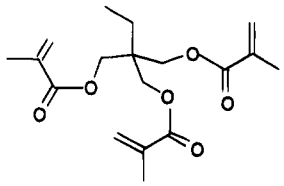
Albright reported the polymerization of a trifunctional methacrylate monomer, trimethylolpropane trimethacrylate (TRIM, **1**) [Abbreviations: TRIM, 2-ethyl-2-(hydroxymethyl)propane-1,3-diol trimethacrylate; AIBN, 2,2′-azobisisobutyronitrile ; XPS, X-ray photoelectron spectroscopy; SEM, scanning electron microscopy; EGDMA, ethylene glycol dimethacrylate] to form a cross-linked, macroporous reactive polymer matrix.[9] More recently, Flodin and co-workers observed that the poly(TRIM) matrix contains residual unreacted double bonds, whose concentration can be controlled by choosing appropriate polymerization parameters such as polymerization time and temperature and the nature and concentration of the solvent (porogen).[10] These polymer-bound double bonds are accessible for subsequent chemical manipulations.[11] Hargitai and co-workers have recently reported the preparation of supports for chromatographic chiral separations by grafting chiral polymer chains onto poly(TRIM) particles.[12]



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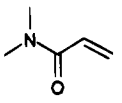

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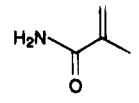

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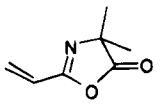

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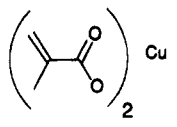

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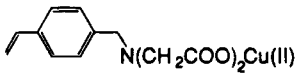
The excellent material properties of macroporous functional copolymer matrices obtained using TRIM as the cross-linker[13] prompted us to evaluate their utility as reactive supports for template polymerization. The high stability of the poly(TRIM) matrix and its relatively low tendency to swell in different solvents suggest that the functionalization could be confined to the porous surface without affecting the bulk mechanical properties. Furthermore, the ease with which methacrylate groups can be copolymerized with different vinyl monomers[14] should enable a variety of functional polymer chains to be anchored to the poly(TRIM) surface.

To evaluate the reactivity of poly(TRIM) toward grafting with functional polymers, poly(TRIM) was modified with four different monomers: *N,N*-dimethylacrylamide (**2**); methacrylamide (**3**); vinylazlactone (**4**) and copper(II) dimethacrylate (**5**). Monomers **2** and **3** would convert the hydrophobic methacrylate surface to a more hydrophilic one, while grafting with **4** would result in a reactive surface amenable to subsequent chemical manipulation.[15] Copolymerization with **5** would lead to a functional matrix possessing metal-coordinating sites. Water absorbency, swelling, accessibility of the new functional groups and changes in surface morphology and other surface properties subsequent to grafting were evaluated. Nonspecific protein adsorption to poly(TRIM) modified with hydrophilic monomers was also investigated. The functional monomers were also copolymerized with TRIM, to allow comparison of matrices with functional groups distributed throughout the bulk polymer to those in which the functional groups are confined to the surface.

The utility of poly(TRIM) as a reactive support for template polymerization was evaluated by grafting polymerizable assemblies of copper(II)−[*N*-(4-vinylbenzyl)imino]diacetate (**6**) and bisimidazole template **7**, with ethylene glycol dimethacrylate (EGDMA) as a cross-linking agent. The ability of the surface-templated polymer to selectively rebind its template bisimidazoles was measured and compared to the bulk-polymerized templated polymers previously reported.[1c]



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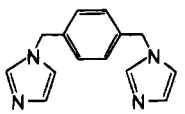

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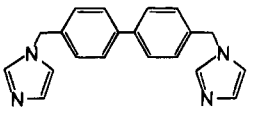

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Results and Discussion

Preparation of Reactive Poly(TRIM) Supports and Grafting with Functional Monomers. Variation of the polymerization parameters enables the material properties of poly(TRIM) to be engineered in a systematic fashion.[11] Polymerization conditions were chosen to yield a macroporous material with desirable morphological characteristics and bearing sufficient residual double bonds for subsequent modification. Polymerization of a 50% (v/v) solution of TRIM in a solvent system of cyclohexane:toluene (70:30 v/v) at 60 °C for 4 h yielded an amorphous, white polymer, which was dried, ground, and sieved.

The different materials prepared by grafting monomers **2**−**5** onto poly(TRIM) are listed in Table 1. Treatment of poly(TRIM) particles with a monomer solution under reduced pressure likely results in diffusion of the monomer along the porous surfaces of the matrix by capillary action. Polymerization of this heterogeneous mixture of functional monomer and poly(TRIM) particles by free radical initiation at 70−80 °C results in grafting of functional polymer chains to the poly(TRIM) surface. The modified polymer was exhaustively extracted to remove unreacted monomers and ungrafted polymers (except some small amount that might be permanently entangled in the porous networks). The amount of material grafted was estimated from the elemental analyses of the copolymers. Thus, the compositions of the polymers with **2**−**4** were determined from the nitrogen content of the modified poly-

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Table 1. Preparation and Specific Surface Areas of Surface-Grafted and Bulk-Copolymerized TRIM-Based Functional Copolymers

polymer	comonomer and (mode of incorporation)	mol % functional monomer in the matrix[a]	specific surface area ($m^2 g^{-1}$)
P-1	poly(TRIM)		285
P-2	**2** (graft)	5.3	215
P-3	**2** (graft)	9.6	212
P-4	**2** (bulk)	5.5	275
P-5	**2** (bulk)	10.2	272
P-6	**3** (graft)	5.5	202
P-7	**3** (graft)	18.5	192
P-8	**3** (graft)[b]	4.3	195
P-9	**3** (graft)[b]	12.6	nd[c]
P-10	**3** (bulk)	6.2	257
P-11	**3** (bulk)	15.5	260
P-12	**4** (graft)	7.3	205
P-13	**4** (graft)	14.2	nd
P-14	**4** (bulk)	4.5	nd
P-15	**4** (bulk)	12.5	nd
P-16	**5** (graft)	4.4	208
P-17	**5** (graft)	14.6	196
P-18	**5** (bulk)	5.3	265
P-19	**5** (bulk)	10.3	260

[a] Estimated from elemental analysis data. [b] Water used as the polymerization medium. [c] Not determined.

mers, and the composition of the polymer with **5** was based on the copper content. The extent of grafting depends on the monomer concentration in the reaction medium, as shown in Figure 1. The grafting process appears to be efficient, resulting in a large fraction of the monomer grafted onto the polymer particles. A significant amount of functional monomer can be grafted to the polymer surface (~25% w/w). Beyond this, the grafting process leaves soluble polymers which are removed by extraction.

Unpolymerized methacrylate residues on the poly(TRIM) surface provide anchoring sites to graft new functional groups. The efficiency of the coupling reaction depends on the accessibility of these double bonds. Previous studies using bromine addition, postpolymerization, and NMR relaxation measurements have demonstrated the accessibility and mobility of these polymer-bound double bonds.[11] The presence of functional polymer chains after exhaustive extraction of these modified polymers with suitable solvents strongly suggests that the functional polymer chains are covalently linked to the poly(TRIM) matrices via the surface-exposed unreacted methacrylate residues.

Macroporous TRIM polymers possess permanent pore structures and significant mechanical rigidity and are less sensitive to the surrounding solvent than lightly cross-linked gel-type polymer resins.[11,16] Reaction in rigid macroporous polymers is controlled by diffusion of the reactants into the pores rather than swelling of the matrix. Thus, in principle, it should be possible to use either aqueous or organic monomer solutions for modification of poly(TRIM). Methacrylamide can indeed be grafted to poly(TRIM) from an aqueous solution, and only slightly less methacrylamide is grafted to the polymer surface in water than in ethanol (see Table 1, entries 6–9). We are interested in the ability of this reactive polymer to graft polymerize in aqueous media for immobilization of water-soluble substrates and other, biological substrates that may be sensitive to organic solvents.

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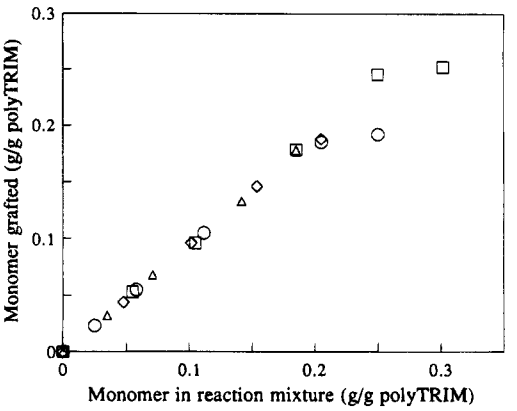

Figure 1. Amount of functional monomer grafted onto poly(TRIM) surface as a function of monomer concentration in the polymerization mixture: monomer **2** (○); **3** (◇) ; **4** (△) and **5** (□).

To compare the physicochemical behavior of the surface-grafted polymers to materials in which the distribution of functional monomers is homogeneous, the TRIM monomer was copolymerized with the respective monomers at similar molar composition, using an appropriate porogen (see Experimental Section for details). The bulk-copolymerized materials, listed in Table 1, were also exhaustively extracted to remove soluble components.

Spectroscopic Analysis of Surface-Grafted Polymers. The functional polymers prepared by grafting to poly(TRIM) were analyzed using infrared (IR), ^{13}C NMR, and X-ray photoelectron spectroscopies (XPS). The presence of residual unpolymerized methacrylate residues is evident from the characteristic peak at 1640 cm^{-1} in the IR spectrum of unmodified poly(TRIM) shown in Figure 2a.[17] Grafting is accompanied by the appearance of new peaks characteristic of the corresponding functional monomers. Thus, the spectrum of methacrylamide-grafted poly(TRIM) (Figure 2b) contains an intense absorption at 1665 cm^{-1} due to the amide carbonyl stretching vibration as well as a broad peak in the region 3200–3500 cm^{-1} corresponding to N–H stretching. Similarly, the IR spectrum of vinylazlactone-modified particles (Figure 2c) reveals the presence of new peaks at 1660 and 1825 cm^{-1} characteristic of the azlactone ring.[18]

Unmodified poly(TRIM) particles were annealed in ethanol at 80 °C for 30 h in the presence of AIBN in order to assess the reactivity of the residual double bonds. The IR spectrum of this polymer, shown in Figure 3, is characteristic of a typical methacrylate polymer, with only the ester carbonyl peak at 1735 cm^{-1}. It appears that complete reaction of the residual double bonds can occur under the conditions of polymerization, in the absence of grafted monomer.

The solid-state ^{13}C NMR spectrum of unmodified poly(TRIM) particles is shown in Figure 4a. Comparison to the ^{13}C NMR analysis of poly(TRIM) reported previously[11] reveals the presence of all expected resonances, including those due to residual double bonds. Resonances due to saturated and unsaturated ester carbonyl carbons appear at 177 and 168 ppm, respectively. The

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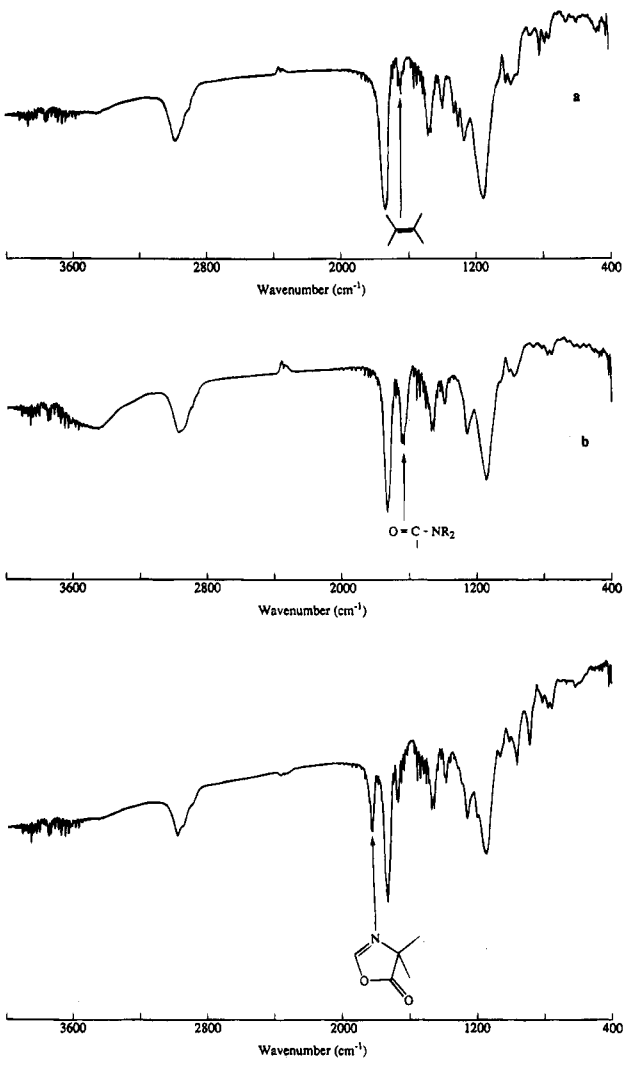

Figure 2. Infrared spectra of (a) macroporous poly(TRIM) bearing residual methacrylate residues (P-1), (b) poly(TRIM) grafted with **3** (P-6), and (c) poly(TRIM) grafted with **4** (P-12).

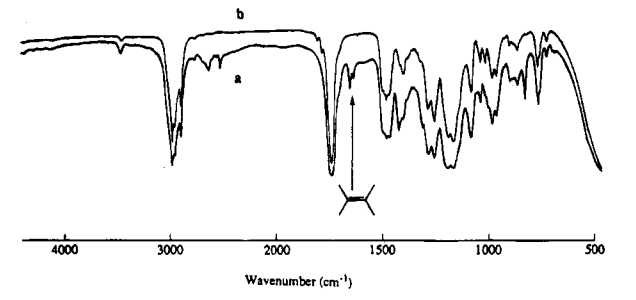

Figure 3. Infrared spectra of (a) macroporous poly(TRIM) bearing residual methacrylate residues (P-1) and (b) the same polymer after annealing at 80 °C for 30 h.

characteristic resonances at 130 (quaternary) and 120 ppm (methylene) also attest to the presence of unpolymerized methacrylate residues. The spectrum of a typical methacrylamide-grafted copolymer (Figure 4b) is similar to that of bulk-polymerized TRIM–methacrylamide (Figure 4c). The specific resonances corresponding to the unpolymerized double bonds are largely gone in both materials. Due to the limited resolution of amide and ester carbonyls in this experiment, no peak characteristic of methacrylamide is evident in either spectrum. However, the reduction in the intensity of the unsaturated ester carbonyl peak, concomitant with

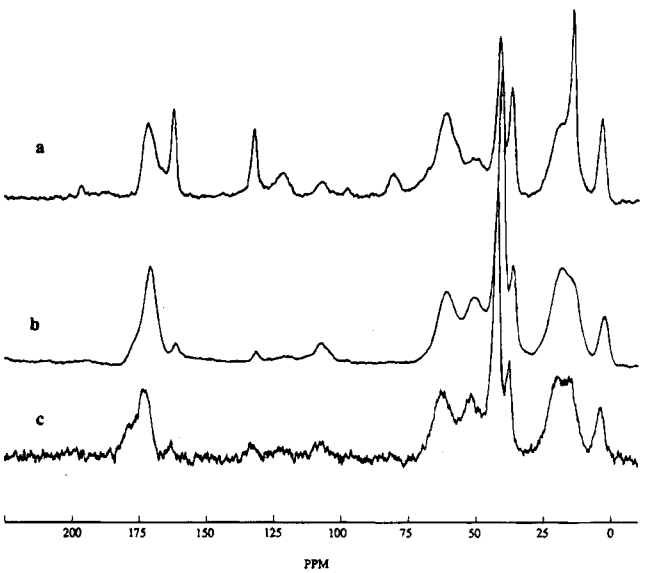

Figure 4. CP-MAS ^{13}C NMR spectra of (a) macroporous poly(TRIM) bearing residual methacrylate residues (P-1), (b) poly(TRIM) grafted with **3** (P-6), (c) bulk-polymerized TRIM-**3** copolymer (P-10).

the increased intensity of the saturated ester carbonyl peak, suggests that the functional polymer chains are grafted to the poly(TRIM) surface.

XPS was used to analyze the chemical nature of the modified surfaces. The advantages of this technique include its element specificity and sensitivity to surface chemical compositions.[19] XPS has been used to characterize a variety of chemically-modified polymer surfaces.[20,21] The observable elemental composition of poly(TRIM) comprises only carbon and oxygen. Accordingly, the XPS spectrum of poly(TRIM) shown in Figure 5a contains only O(1s) and C(1s) peaks at 531 and 290 eV, respectively. (The small peaks at 102 and 153 eV, characteristic of Si(2p) and Si(2s), are probably due to contamination by the silicon grease used for vacuum sealing. This is evidently washed away during subsequent treatment, as these peaks are not seen in the grafted samples.) Because the functional monomers **2**–**5** contain elements (i.e., nitrogen and copper) not present in the original poly(TRIM) polymer, the specific peaks in the grafted copolymers corresponding to these elements indicate the presence of functional polymer chains on the surface. The XPS spectrum of a typical copolymer obtained by grafting with methacrylamide (Figure 5b) shows the N(1s) peak at 395 eV, in addition to the O(1s) and C(1s) peaks. The spectrum of the copper(II) dimethacrylate-grafted copolymer in Figure 5c reveals the typical Cu(2p) doublet peaks at 932 and 952 eV.

The elemental composition of the polymer surface can be obtained from the XPS analysis. The nitrogen content of the graft copolymer with monomer **3** (P-6, Table I), recorded at a takeoff angle of 90°, was found to be 4.0%. Decreasing the takeoff angle to 30° increased the intensity of the N(1s) peak of copolymer P-6

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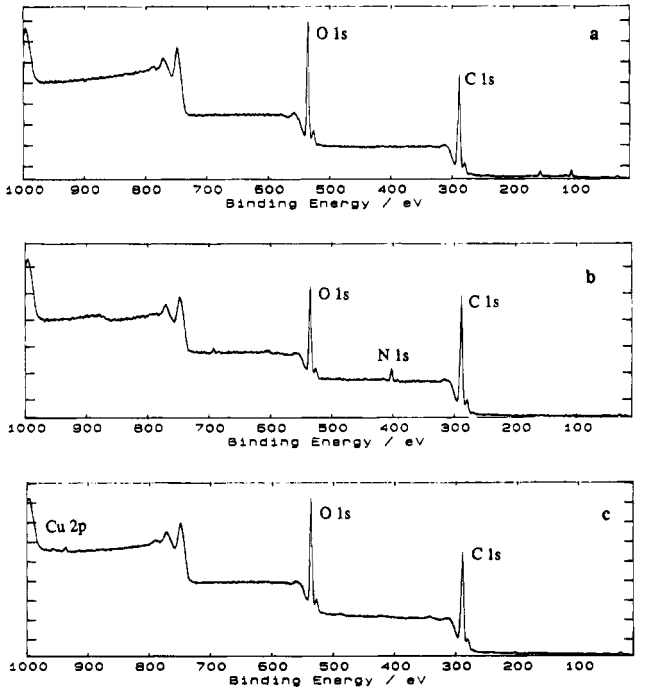

Figure 5. X-ray photoelectron spectra of (a) macroporous poly(TRIM) bearing residual methacrylate residues (P-1), (b) poly(TRIM) grafted with **3** (P-6), and (c) poly(TRIM) grafted with **5** (3.2 mol %).

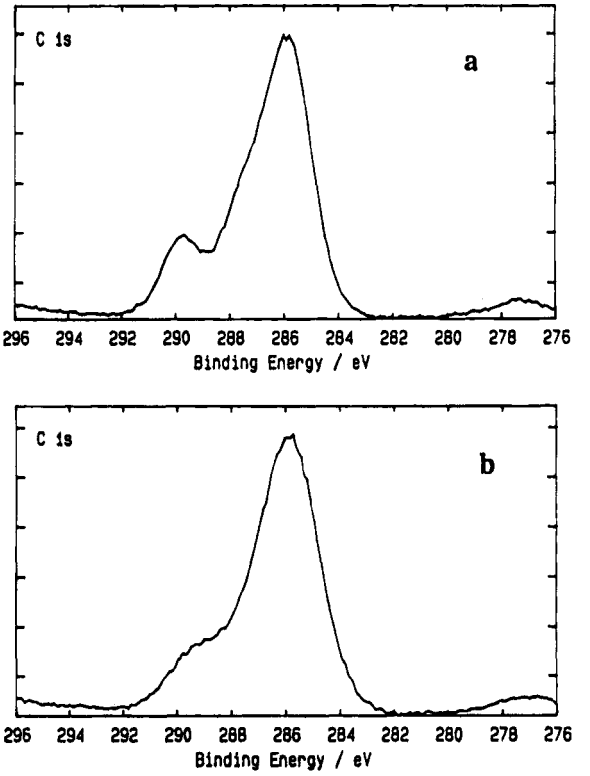

Figure 6. X-ray photoelectron spectra of the C(1s) region of (a) poly(TRIM) (P-1); (b) poly(TRIM) grafted with **3** (P-6).

to correspond to a nitrogen content of 4.4% (data not shown). The overall nitrogen content of this material was found to be 3.8% by chemical analysis. Although small, these variations in the angle-dependent measurements of the nitrogen content are precise, reproducible, and consistent with the location of grafted chains toward the surface of the poly(TRIM) particles.

An expanded spectrum of the C(1s) peak of poly(TRIM), shown in Figure 6a, shows two distinct peaks, centered at 286 and 290 eV. These peaks correspond to backbone and other hydrocarbon carbon and ester carbonyl carbon atoms, respectively. The expanded C(1s) region of the methacrylamide-grafted sample is shown in Figure 6b. The broadening of these peaks compared to the precursor polymer can be attributed to an additional amide carbonyl C(1s) peak at 289 eV that overlaps the ester carbonyl carbon peak.

Morphologies of Surface-Grafted Polymers. Anchoring new polymer chains by chemical grafting changes the morphology of the poly(TRIM) particles. The unmodified poly(TRIM) particles are macroporous and possess high surface area (285 $m^2\ g^{-1}$), as indicated in Table 1. Further information on the morphology of this material can be obtained by scanning electron microscopy (SEM), which reveals the porous texture of the poly(TRIM) (Figure 7a). Matrices prepared by simultaneous copolymerization of TRIM with the functional monomers possess surface areas similar to the poly(TRIM) matrix (Table 1). SEM studies of these polymers also show that the macroporous structures are similar, as seen in the micrograph of the bulk copolymer of TRIM with methylacrylamide (Figure 7b).

Grafting functional monomers to poly(TRIM) decreases the specific surface area (Table 1). Electron micrographs of the graft copolymers obtained using functional monomers **2** and **3**, shown in Figure 7c,d, reveal significant changes in the surface morphology upon grafting. The grafted materials have a less open surface texture, with decreased pore volumes, due to incorporation of the new grafted polymer chains in the porous spaces of the poly(TRIM) matrix. This reinforcement leads to more compact structures, while retaining the overall macroporous morphology.

Physicochemical Characteristics of Bulk-Polymerized and Surface-Grafted Poly(TRIM) Particles. The surface-grafted metal-complexing polymer made with monomer **5** and the corresponding macroporous polymers with homogeneous distributions of metal-complexing sites were extracted with a strong Cu(II) chelator, ethylenediaminetetraacetic acid (EDTA). As shown in Table 2, nearly 90% of the total copper could be removed from the surface-grafted polymer in a single extraction step. On the other hand, bulk polymerized samples release only ~65% of the total copper under similar extraction conditions. While the metal-complexing sites in the surface-grafted material are accessible to EDTA, bulk copolymerization leads to a distribution of metal sites, some of which, presumably in the interior of the matrix, are inaccessible to EDTA. The nonpolar nature of the matrix interior may limit the partitioning of the highly polar chelating agent. This behavior parallels our earlier observations with metal-complexing templated polymers. EDTA was unable to completely remove the copper ions from the bulk-polymerized EGDMA matrix, while a lipophilic chelating agent (triazacyclononane) was effective.[1c]

Bulk-polymerized and surface-grafted matrices obtained using hydrophilic monomers **2** and **3** do not swell to any significant extent in a nonsolvent, hexane. Both the bulk-polymerized and surface-grafted polymers swell appreciably in water, as indicated in Table 3. The swelling is more pronounced for the bulk-copolymerized materials. For example, a surface-grafted polymer

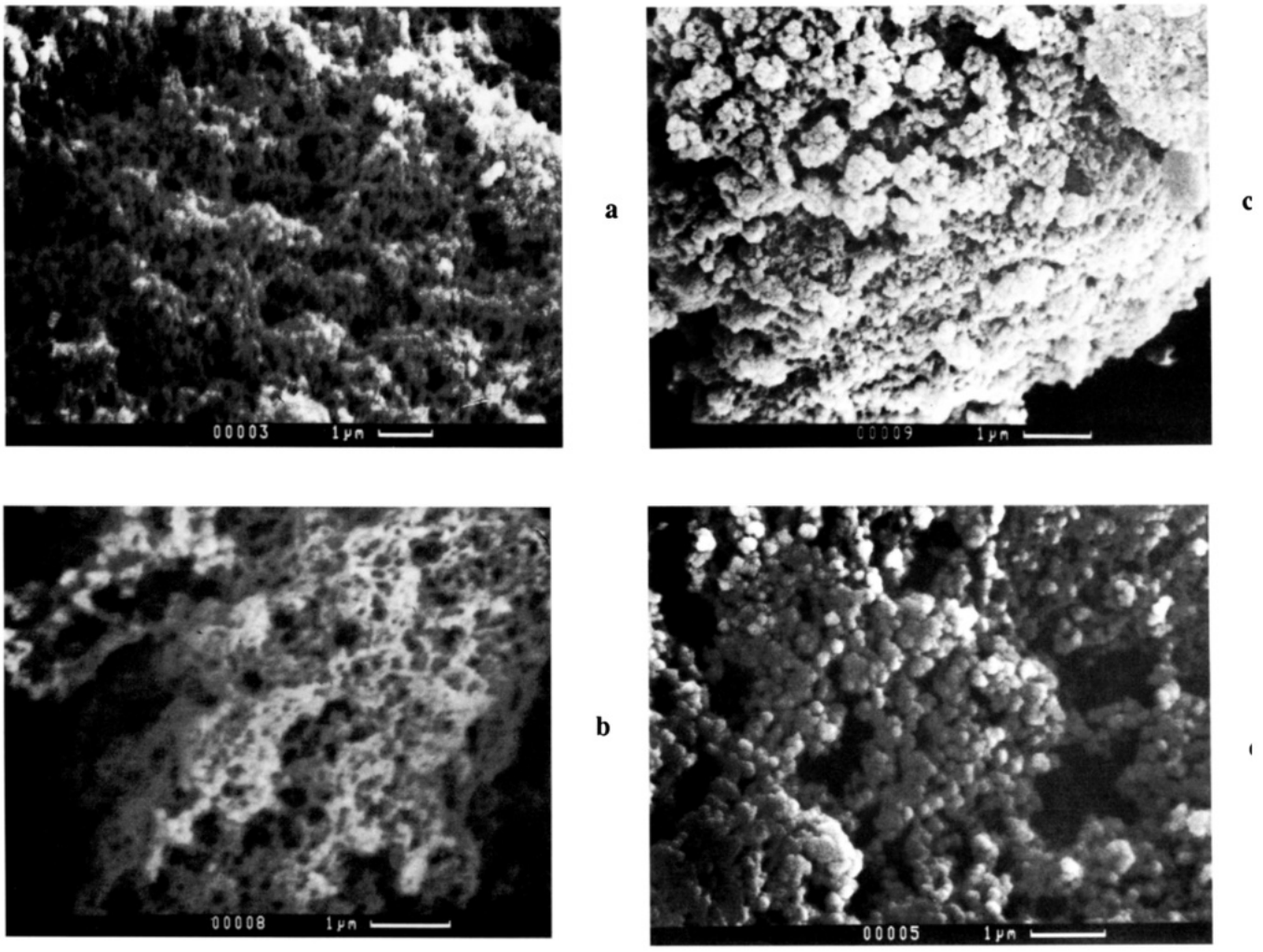

Figure 7. Scanning electron micrographs of (a) poly(TRIM); (b) bulk polymerized TRIM-**3** copolymer (P-10); (c) poly(TRIM) grafted with **2** (P-2); (d) poly(TRIM) grafted with **3** (P-6).

Table 2. Copper Removal from TRIM Copolymers by EDTA Treatment

polymer code[a]	Cu(II) (mmol/g) in the copolymer	recovery of Cu(II) (mmol/g) from the copolymer[b]
P-16	0.13	0.11
P-17	0.45	0.41
P-18	0.16	0.10
P-19	0.38	0.25

[a] Polymer type from Table 1. [b] Amount of copper released from the matrix by EDTA treatment alone.

Table 3. Swelling of Surface-Grafted and Bulk-Copolymerized TRIM-Based Copolymers in Water

polymer code[a]	% swelling in water
P-2	15
P-3	20
P-5	45
P-6	20
P-7	35
P-11	75

[a] Polymer type from Table 1.

containing 18 mol % methacrylamide (P-7) swells to 135% of its dry volume in water, while the corresponding bulk-polymerized matrix containing 15 mol % methacrylamide (P-11) swells to 175% of its original volume. Distribution of the hydrophilic comonomers across the polymer matrices permits greater water penetration and swelling for the bulk-polymerized materials, while these effects are probably confined to the surface of the grafted copolymers. While grafting with methacrylamide fills the microporous surface regions of the parent poly(TRIM) polymer, the bulk-copolymerized supports possess a porous texture similar to that of poly(TRIM). Copolymerization with a nominal amount of the hydrophilic comonomer does not dramatically influence the polymer morphology. Interaction with water is anticipated to bring about structural rearrangements of the hydrophilic polymer networks, which can be manifested in their morphologies. Furthermore, morphological changes are likely to differ for different structural architectures.

SEM micrographs obtained of the bulk-copolymerized polymer of TRIM with **3** and the corresponding surface-grafted support after swelling both materials in water are shown in Figure 8. A comparison of the surface-functionalized material before (Figure 7d) and after swelling in water (Figure 8a) shows little change in overall macroporous morphology (Figure 7d). In contrast, swelling the bulk-polymerized support in water (Figure 8b) greatly increases the size of the individual globules and agglomerates and causes a large increase in the interglobular spaces, compared to its dry precursor (Figure 7b). These observations suggest that the porous morphologies are sensitive to the solvent, but the magnitude of this change depends on the degree to

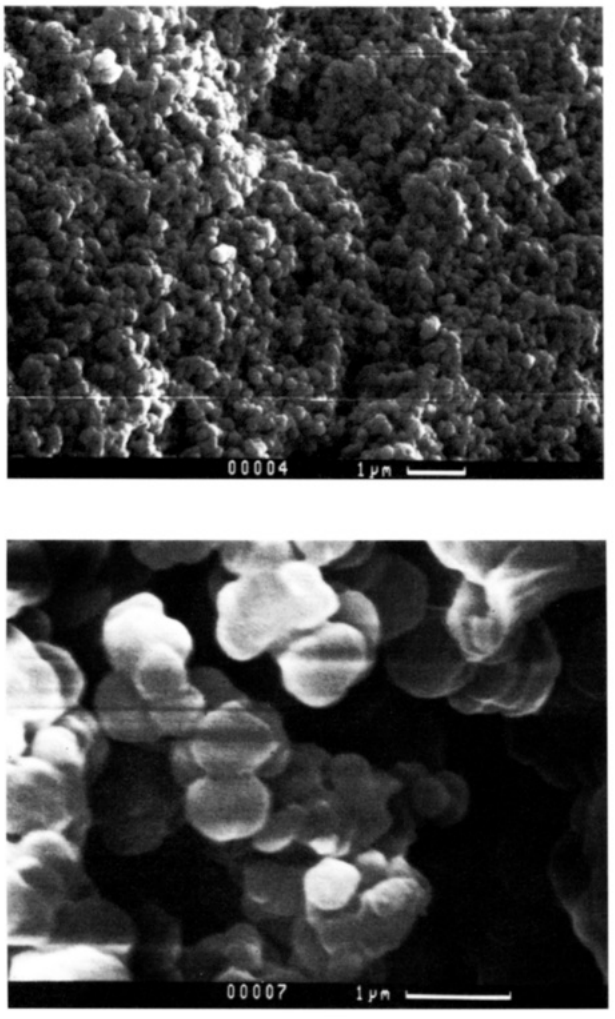

Figure 8. Scanning electron micrographs of water-equilibrated samples of (a) poly(TRIM) grafted with **3** (P-6); (b) bulk-polymerized TRIM-**3** copolymer (P-10).

which solvent can penetrate into the polymer matrix (i.e., degree of swelling). The changes are nominal for the surface-modified matrix, the water penetration being confined to the few grafted layers. The greater solvent penetration and overall swelling of the bulk-copolymerized material results in more significant morphological rearrangement.

Protein Adsorption to Modified Poly(TRIM) Surfaces. Minimizing nonspecific protein adsorption is desirable for materials destined for bioseparations. We therefore investigated the adsorption of bovine serum albumin (BSA) to modified and unmodified poly(TRIM) surfaces. Adsorption measurements were carried out on (i) unmodified poly(TRIM), (ii) poly(TRIM) grafted with methacrylamide, and (iii) bulk-polymerized TRIM–methacrylamide, at pH 7.2 and varying ionic strengths. Adsorption isotherms obtained in 1 M NaCl (Figure 9) indicate that grafting methacrylamide to the poly(TRIM) surface serves to reduce protein binding, although the residual adsorption is still significant. The bulk- and graft-copolymerized matrices bind similar levels of protein. Decreasing the salt concentration decreases BSA adsorption to the grafted polymer (data not shown), behavior that is characteristic of adsorption involving hydrophobic interactions.

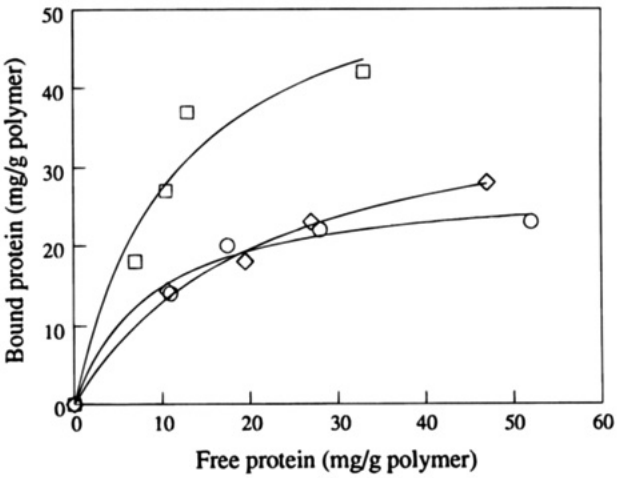

Figure 9. Adsorption of bovine serum albumin (1 M NaCl, pH 7.2) on poly(TRIM) (□); poly(TRIM) grafted with **3** (P-9) (○); and bulk polymerized TRIM-**3** copolymer (P-11) (◇).

Generation of Specific Binding Sites by Surface-Grafting Template Polymerization. The residual methacrylate groups on poly(TRIM) can be utilized to graft a polymerizable monomer:template assembly and cross-linking agent for surface template polymerization. Similar to our previous template polymerization study with bulk-polymerized materials,[1] copper(II) [*N*-(4-vinylbenzyl)imino]diacetate (**6**) was used as the metal-complexing monomer and 1,4-bis(imidazol-1-ylmethyl) benzene (**7**) as the template. The polymerizable monomer–template assembly, generated by combining methanol solutions of **7** and **6** (mole ratio **6**:**7** = 2:1), was polymerized with the TRIM matrix and ~40 mol % EGDMA (for details, see experimental section). After polymerization, the light blue polymer was extracted exhaustively with hot methanol to remove any soluble components. Following our earlier procedures,[1c] treatment of this polymer with acidified aqueous methanol followed by EDTA enabled removal of the bisimidazole template and copper ions. A control polymer with a random distribution of metal centers was prepared under similar polymerization conditions, using 1-benzylimidazole as the template.

Copper ions could be removed to a large extent (>90%) from both these surface-grafted polymers with a single EDTA treatment. The nonpolar chelating agent, 1,4,7-triazacyclononane, necessary to completely remove the copper from the bulk-copolymerized materials, is not required for the surface-grafted materials. Reloading with copper is achieved by treating the metal-free polymers with $CuCl_2$, followed by thorough washing to remove unbound copper ions.

The degree to which the placement of metal centers in the polymer complements the structure of the template should be reflected in preferential binding. A study of selective substrate binding by bulk-polymerized metal-complexing templated polymers has been reported.[1c] Analogous competitive binding experiments were carried out for the surface-grafted materials using substrate **7** and a second bisimidazole derivative, **8**. The surface-grafted templated polymer prepared using **7** as the template binds its own template bisimidazole preferentially, with a separation factor ($\alpha_{7/8}$) of 1.30. The control polymer, in contrast, was unable to distinguish the two bisimidazole substrates ($\alpha_{7/8} \sim 1.0$). The selectivity of the surface-grafted polymer is similar to

selectivities obtained for bulk-polymerized materials.[1c] Thus, the poly(TRIM) matrix provides a support suitable for surface template polymerization.

Conclusions

By controlling the polymerization conditions it is possible to obtain macroporous poly(TRIM) with surface-accessible polymerizable double bonds. These reactive double bonds can be used to link functional polymer chains to the surface of the solid support by polymerization in the presence of appropriate functional monomer. Compared to the macroporous copolymers with a homogeneous distribution of functional groups, confining the functional polymer chains to the surface of the matrix enhances their accessibility for substrate binding. Moreover, as the modifications are confined to the surface regions, solvent effects influencing the functional polymer chains do not compromise the overall matrix integrity. Thus functional polymer surfaces possessing desirable material properties can be obtained. In particular, the poly(TRIM) reactive surface appears to be well-suited for surface template polymerization in order to create highly selective adsorbents for chromatographic separations.

Experimental Section

Materials. Copper(II) dimethacrylate was synthesized following the procedure of Yici et al.[22] Synthesis of compounds **6**–**8** have been reported previously.[1c] Methacrylamide and vinylazlactone were obtained from Monomer-Polymer Inc. All other reagents were obtained from Aldrich. Reagents and the solvents were purified following standard methods. 2,2′-Azobisisobutyronitrile (AIBN) was recrystallized from ethanol prior to use.

Analysis Procedures. Elemental analyses were performed at Galbraith Laboratories, Knoxville, TN. Melting points were determined on a Buchi melting-point apparatus. IR spectra were recorded in the form of clean KBr pellets using a Perkin-Elmer 1600 FTIR spectrophotometer. Electronic absorption measurements were carried out with a Milton-Roy Array 3000 spectrophotometer. Solid-state CP-MAS ^{13}C NMR spectra were recorded on a Bruker MSL-200 spectrometer operating at 50.3 MHz. Chemical shifts were calibrated by external reference to aromatic carbon of hexamethyl benzene (132.1 ppm relative to TMS). Specific surface areas and pore volumes of the polymers were determined from nitrogen adsorption measurements using an Omnisorp 100 analyzer. Electron micrographs were obtained with a Cam Scan series 2 scanning electron microscope after vacuum coating of the samples with gold. X-ray photoelectron spectra (XPS) of the powder polymer samples were obtained with a VG Scientific Ltd. ESCALAB MK2 electron spectrometer equipped with a VG 5250 data system and x, y, z axes and θ computer controlled translation stage. The sprectrometer was operated at a base pressure of about 8.0×10^{-11} Torr. The unmonochromatized Mg Kα X-ray source (1253.60 eV) was operated at 15 kV and 20 mA (300 W) and bandpass was set for a resolution of 0.8 eV. Spectra were collected in CAE mode at the appropriate takeoff angles, with the angle defined as that between the sample surface plane and the analyzer axis. Charge compensation was done using a flood gun and referencing of the peaks was carried out by considering the binding energy of hydrocarbon type carbon as 286.0 eV. The samples were dried at 60 °C under high vacuum for 24 h prior to analysis.

Polymerization Reactions and Workup. *Preparation of poly(TRIM) support:* In a 250 mL round bottom flask, 10 g of TRIM monomer was dissolved in 25 mL of toluene:cyclohexane (30:70 v/v). To this 100 mg of AIBN was added, and the reaction mixture was bubbled with argon gas for 2 h. The sealed flask was kept at 55 °C for 4 h with gentle stirring. The polymer thus obtained was cooled to room temperature, broken into small pieces, and extracted with 250 mL of methanol for 8 h at 37 °C in a shaker bath to remove any unreacted monomer. After filtering, the polymer was dried under vacuum to constant weight, ground, and sieved. Particles in the size range 38–63 μm were used for subsequent experiments.

*Preparation of bulk copolymers of TRIM with functional monomers (**2**–**4**).* A typical procedure is described using **2** as the functional monomer. 4 g of TRIM , 0.6 g of **2**, and 46 mg of AIBN were dissolved in 10 mL of toluene:dioxane (80:20 v/v). The reaction mixture was bubbled with argon for 1 h and polymerized at 70 °C for 12 h and at 80 °C for 15 h. After cooling to room temperature, the polymer was washed with ethanol and dried under vacuum. It was subsequently ground, sieved to the appropriate particle size (63–38 μm), extracted with hot methanol overnight, and dried to constant weight under vacuum at 50 °C.

*Preparation of bulk copolymer of TRIM with **5**:* In a typical experiment, a solution of 4 g of TRIM and 50 mg of AIBN dissolved in 6 mL of ethanol was added to 5 mL of ethanolic solution of monomer **5** (0.8 g). After the reaction mixture was bubbled with argon, polymerization and subsequent work up were carried as described above.

Comonomer grafting on poly(TRIM) surface: The general procedure for grafting is described using **2** as the comonomer. Typically, a 50 mL round-bottom flask with a sidearm (outlet) containing 1 g of macroporous poly(TRIM) particles was evacuated for 5 h with gentle stirring. After closing the vacuum connection, a solution of 0.25 g of **2** and 15 mg of AIBN in 5 mL of dioxane was injected slowly (with the system still under vacuum). The resulting mixture was allowed to stir gently under vacuum for 18 h, during which time the monomer solution diffuses inside the pores of the polymer support. After purging with argon for 3 h, the flask was kept at 60 °C for 12 h, at 70 °C for 6 h, and finally at 80 °C for 6 h to ensure complete polymerization. The polymer was cooled to room temperature, washed with 30 mL of methanol, and filtered. The solid residue was subsequently Soxhlet extracted with refluxing methanol for 24 h to remove ungrafted soluble polymers. After filtering, the polymer was dried to constant weight under vacuum at 50 °C.

Copper removal: Typically 0.5 g of polymer was suspended in 30 mL of 0.1 M aqueous EDTA (pH 7.0) and was kept in a shaker bath at 37 °C for 36 h. After the polymer was filtered and thoroughly washed, the amount of copper extracted to the aqueous phase was estimated spectrophotometrically.[1c]

Polymer swelling: The swelling measurements of these polymer particles were carried out following a literature procedure.[23] A given volume of dry polymer (typically 2 cm^3) was transferred to a 10 mL graduated centrifuge tube, graduated to each tenth of a milliliter. The polymer was allowed to pack properly with the help of a vortex vibrator, and the exact dry volume was noted. Excess solvent (approximately 5 times) was added, and any trapped air bubbles were removed by vibration. The polymer was kept at 37 °C in a shaker bath for 24 h. After centrifugation, the final volume of the solvent swollen polymer was noted. Values of the dry and swollen volumes of the polymer were used to calculate percent swelling of the polymer (percent swelling = 100 × change in volume/volume dry polymer). The values reported are an average of three measurements which have a mutual variation of less than 5%.

Template Polymerization by Grafting Polymerizable Monomer–Template Assembly onto Poly(TRIM). To a stirred solution of 0.6 g of monomer **6** in 5 mL of methanol, 0.21 g of template **7** in 5 mL of methanol was added slowly. After this stirred for 30 min, 0.25 g of EGDMA and 25 mg of

(22) Yici, G.; Yaoyu, W.; Ying, Z.; Qizhen, S. *Polyhedron* **1991**, *10*, 1893–1895.

(23) Shea, K. J.; Stoddard, G. J.; Shavelle, D. M.; Wakui, F.; Choate, R. M. *Macromolecules* **1990**, *23*, 4497–4507.

AIBN were added, and the solution was bubbled with a slow stream of N_2 for 30 min. This monomer solution was injected slowly to 3 g of macroporous poly(TRIM) in a 100 mL round-bottom flask (evacuated as described above). The resulting suspension was allowed to stir under vacuum for 18 h. Polymerization and work up was similar to the procedure described above for grafting of other functional monomers. A control polymer was prepared in a similar manner, using 1-benzylimidazole instead of **7** as the template. Templates and metal ions were removed, and metal reloading and substrate binding studies were performed following procedures described previously.[1c]

Protein Adsorption. Bovine serum albumin (BSA) (Sigma grade, fraction V, powder, 96–99%) was used for protein adsorption experiments. Typically, 0.5 g of polymer particles were washed with deionized water and equilibrated with the binding buffer (sodium phosphate, pH 7.2) in a 10 mL centrifuge tube for 12 h. In case of unmodified poly(TRIM), the polymer was first washed with 9:1 (v/v) water:ethanol, followed by deionized water before equilibration with the binding buffer. The polymer suspension was centrifuged, and the supernatant was removed. A predetermined amount of the protein (dissolved in 5 mL of the same buffer) solution was added to the wet polymer. The protein–polymer suspension was equilibrated at 25 °C for 2 h on a rotator. The sample was subsequently spun at 10 000 rpm for 15 min, and the supernatant was transferred to a fresh tube. The polymer sample was washed with the binding buffer (2 × 2 mL) and was combined with the first supernatant. The concentration of BSA in the combined washings (in duplicate experiments) was determined using UV spectrophotometry by measuring the absorbence at 280 nm (by comparison to calibration curve). The amount of protein adsorbed to the polymer surface was estimated from a mass balance on the total protein added and the total protein in the combined washes.

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