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Synthesis of Hetero-Telechelic α,ω Bio-Functionalized Polymers

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Reversible addition−fragmentation chain transfer (RAFT) polymerization was used to synthesize poly[diethylene glycol monomethylether methacrylate] (PDEGMA) (M_n = 6250 g/mol, PDI = 1.14) with a pentafluorophenyl (PFP) activated ester and a dithioester end group. The hormone thyroxin (T4) was quantitatively attached to the PFP activated ester α end group via its amino group. The ω-terminal dithioester was not harmed by this reaction and was subsequently aminolyzed in the presence of *N*-biotinylaminoethyl methanethiosulfonate, yielding a polymer with a thyroxin and a biotin end group with very high heterotelechelic functionality. The polymer was characterized by ^{1}H, ^{13}C, and ^{19}F NMR, UV−vis, and IR spectroscopy and gel permeation chromatography. The thyroxin transport protein prealbumin with two thyroxin binding sites and streptavidin, which has four biotin binding sites, was conjugated using the biotarget labeled polymer, resulting in the formation of a protein−polymer network, confirming the heterotelechelic nature of the polymer. Polymer−protein microgel formation was observed with dynamic light scattering. To realize a directed protein assembly, prealbumin was immobilized onto a surface, exposing one of its two thyroxin binding groups and thus allowing the conjugation with the thyroxin α end group of the heterotelechelic polymer. The biotin ω end group of the attached polymer layer enabled the subsequent immobilization of streptavidin, yielding a defined multilayer system of two proteins connected with the synthetic polymer (efficiency of streptavidin immobilization 81% based on prealbumin). Without the polymer, no streptavidin immobilization occurred. The layer depositions were monitored by surface plasmon resonance. The synthetic approach of combining PFP activated esters with functional MTS reagents presents a powerful method for obtaining well-defined heterotelechelic (bio-) functionalized polymers.

Introduction

The use of synthetic polymers in medicine and biotechnology has found applications and research interests in many fields,[1–5] such as improving the activity and stability of proteins[6,7] or for drug delivery purposes.[8–10] Besides the use of poly(ethylene glycol) (PEG) for stealth systems,[11] "smart" polymers such as poly[*N*-isopropyl acrylamide] or poly[ethylene glycol methacrylates] with PEG side chains are also employed to create stimulus responsive polymer−protein conjugates.[12–18] Especially the end groups of a polymer are the focus for biofunctionalization, as they allow for a directed one-to-one attachment.[15,16,19] In addition to methods of covalently connecting synthetic materials and proteins, biology offers the possibility of strong noncovalent bonds based on the bioaffinity of certain proteins toward specific targets. This molecular recognition is especially exploited on surfaces for the development of biosensors, chip-based bioassays, or cell adhesion studies.[20] Polymer brushes,[21] available through surface-initiated controlled radical polymerization techniques, may prevent unspecific binding of proteins to surfaces and also offer the possibility of specific protein or cell immobilization through postmodifications.[22–25] Due to the strong affinity between streptavidin and biotin,[26–28] streptavidin functionalized surfaces are used as versatile platforms for further immobilization and investigation of biomolecules such as DNA or enzymes.[29–31] For such purposes, often multilayers of biological components are constructed, requiring building blocks with two specific binding sites.[32–35] It would be of considerable interest to include telechelic synthetic polymers as heterodimeric cross-linkers in these constructions, exploiting the advantages of polymers, such as solubility, flexible functionalization, or stimulus responsive behavior. However, due to the lack of synthetic possibilities in the past, such efforts were limited.

Controlled radical polymerization techniques offer, besides a precise control over molecular weight, the unique possibility to define chain end groups of a polymeric chain. While several approaches for the biofunctionalization of one end group of a polymer have been published,[15,16,19,36,37] there is still a growing synthetic challenge to produce well-defined narrowly distributed heterotelechelic polymers carrying the same[38,39] or two different biofunctionalities.[40,41] Reversible addition−fragmentation chain transfer (RAFT) polymerization[42] is a very promising method[43] as it allows for an introduction of functional or reactive α end groups via functionalization of the leaving group (R) of the chain transfer agent (CTA).[15,19,38–45] A dithioester or trithiocarbonate is retained as ω end group and may also carry a functionality that was installed into the CTA.[40,43] The dithioester or trithiocarbonate link between an ω (bio-) functionality and the polymer chain is, however, very susceptible toward chemical decomposition.[46–50] Electron deficient dithioesters are suitable for hetero Diels−Alder conjugations with very high conversions.[51–53] A diazo-initiator exhibiting a protected maleimide has been utilized to replace the trithiocarbonate end group with a bioreactive functionality.[39,41] However, the deprotection step via retro Diels−Alder reaction required harsh conditions that caused cleavages of ester bonds.[41] Poly[(meth)acrylates], such as poly(ethylene glycol methacrylates), are thus not eligible for this approach. A different method is to perform an aminolysis of the terminal

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dithioester in the presence of a reagent that will selectively react with the emerging terminal thiol-group.[37,54,55] Boyer et al. presented a versatile way to introduce a thiol-reactive ω end group through aminolysis in the presence of dipyridyldisulfide that could subsequently be used for bioconjugation.[36]

Herein, we report on the synthesis of poly[2-(2-methoxyethyloxy)ethyl methacrylate], also known as poly(diethylene glycol monomethylether methacrylate) (PDEGMA),[12] a biocompatible polymer, carrying two different terminal biotargets, each capable of binding specifically to a certain protein. RAFT polymerization in combination with two orthogonal end-group functionalization routes, that is, activated pentafluorophenyl ester α end groups[56] and modification of the dithioesters using functionalized methane thiosulfonates, is expected to open a versatile pathway toward heterotelechelic biofunctionalized polymers with very high end group conversions. As biotin binds very specifically to streptavidin[26–28] and the hormone thyroxin is recognized and bound by its transport protein prealbumin (transthyretin),[57,58] biotin and thyroxin were used as model targets. The α,ω biotarget labeled polymer was used for heterodimeric protein conjugation both in solution and through directed surface immobilization.

Experimental Section

Materials and Methods. Biotin-MTS (*N*-biotinylaminoethyl methanethiosulfonate) was purchased from Toronto Research Chemicals. All other materials were obtained from Sigma-Aldrich or Acros and used as received. Phosphate buffered saline (PBS) contained 8.0 g/L of NaCl, 0.2 g/L of KCl, 1.44 g/L of Na_2HPO_4, and 0.24 g/L of KH_2PO_4 and was sterilized by boiling.

Surface plasmon resonance (SPR) measurements were performed on 0.1 mg/mL aqueous buffer solutions at 19 °C in a self-built cell of 0.5 mL volume using a $\Theta/2\Theta$ setup, a 632 nm laser, and a photodiode. Glass slides were coated with 1.5 nm of chromium and 50 nm of gold by evaporation. SPR scans were fitted using WINSPALL, assuming a refractive index of all organic materials of 1.5.

^{1}H, ^{13}C, and ^{19}F NMR spectra were measured on a 400 MHz instrument by Bruker on $CDCl_3$ solutions at room temperature.

Dynamic light scattering was measured on a Malvern Zetasizer Nano in a low volume glass cuvette (45 μL) at 20 °C on 0.166 g/L buffered aqueous solutions at an angle of 90°.

Static light scattering (SLS) was performed on methanol solutions with an ALV-SP86 goniometer and a HeNe laser (632.8 nm wavelength) and was used to determine the weight-average molecular weight.

UV−vis absorbance spectra were recorded on a UV 2102 PC instrument by Shimadzu on $CHCl_3$ solutions. The absorbance of the dithioester of PFP-PDEGMA-DTE (**1**) at 302 nm ($\varepsilon = 9.31 \times 10^3$ L mol^{-1} cm^{-1}) was used to calculate the molecular weight of the polymer.[56] Molecular weights of polymers **2** and **3** were based on the weight of polymer **1**, assuming quantitative end group conversions. The concentrations of the solutions for UV−vis spectra plotted in Figure 3 were 150 μM for polymer **2** and thyroxin. The absorbance spectra of polymer **1** and polymer **3** plotted in Figure 3 were measured on 160 μM (**1**) and 142 μM (**3**) solutions, respectively, and normalized to a concentration of 150 μM before plotting.

IR spectra were measured on a Nicolet Specac ATR-FT instrument on pure materials.

Gel permeation chromatography (GPC) was performed on 2 mg/mL THF solutions on MZ-Gel SDplus columns to determine the polystyrene equivalent molecular weight and the polydispersity index M_w/M_n.

Dialysis was performed on cellulose membranes with a molecular weight cutoff (in water) of 12−14 k with methanol as solvent.

α-Pentafluorophenyl Ester, ω-Dithioester Poly[diethyleneglycol monomethylether methacrylate] (PFP-PDEGMA-DTE, 1). The starting polymer was prepared according to a literature procedure.[56] M_n (GPC, PS-equivalent) = 8.0 kg /mol; PDI (GPC) = 1.14; M_n (UV−vis) = 6250 kg/mol; M_w (SLS) = 6900 g/mol. ^{1}H NMR ($CDCl_3$, 400 MHz, δ/ppm) 7.83 (m, 2H, *o*-C_6H_5-CS_2-), 7.48 (m, 1H, *p*-C_6H_5-CS_2-), 7.32 (m, 2H, *m*-C_6H_5-CS_2-), 4.07 (bs, C(O)OC$\underline{H}_2$-), 3.65, 3.59, 3.52 (bs, C(O)O-$CH_2C\underline{H}_2$-O-$C\underline{H}_2C\underline{H}_2$-O-$CH_3$), 3.36 (bs, -O-C$\underline{H}_3$), 2.85 (m, 2H, C$\underline{H}_2$-C(O)O$C_6F_5$), 2.0−1.2 (m, -C$\underline{H}_2$-C($CH_3$)(COO-)), 1.4−0.7 (m, -$CH_2$-C(C$\underline{H}_3$)(COO−)); ^{13}C NMR ($CDCl_3$, 100 MHz, δ /ppm) 177.3 (-CH_2-C(CH_3)(C$\underline{O}$O-)), 132.4 (*p*-C_6H_5-CS_2-), 128.4 (*m*-C_6H_5-CS_2-), 126.6 (*o*-C_6H_5-CS_2-), 71.9, 70.5, 68.4 (C(O)O-$CH_2\underline{C}H_2$-O-$\underline{C}H_2\underline{C}H_2$-O-$CH_3$), 63.8 (C(O)O-$\underline{C}H_2$-), 59.0 (-O-$\underline{C}H_3$), 54.4 (-$\underline{C}H_2$-C($CH_3$)-(COO-)), 45.0, 44.6 (-$CH_2$-$\underline{C}$($CH_3$)(C$\underline{O}$O-)), 28.8 (-$\underline{C}H_2$-COO-$C_6F_5$), 16.2, 18.6 ($CH_2$-C($\underline{C}H_3$)(COO-)); ^{19}F NMR ($CDCl_3$, 376 MHz, δ /ppm) −153.0 (2F), −157.9 (1F), −162.4 (2F).

α-Thyroxin, ω-Dithioester Poly[diethyleneglycol monomethylether methacrylate] (T4-PDEGMA-DTE, 2). To 5 mL of a 13.3 mM solution of PFP-PDEGMA-DTE (66.5 μmol) in DMF, 51.7 mg (66.5 μmol) of thyroxin and 18.6 μL (133 μmol) of triethylamine were added. After 3 h, the thyroxin had dissolved and the mixture was stirred overnight at room temperature. After removing the solvent in vacuum, the residue was dissolved in methanol and was purified by dialysis in methanol for 2 days with solvent changes three times a day. The yield of T4-PDEGMA-DTE was 42%. M_n (GPC, PS-equivalent) = 7.4 kg /mol; PDI (GPC) = 1.15; M_n (UV−vis) = 6850 kg/mol; λ_{max} (UV−vis) = 302 nm. ^{1}H NMR ($CDCl_3$, 400 MHz, δ/ppm) 7.86 (m, 2H, *o*-C_6H_5-CS_2-), 7.70 (m, 2H, HO-$C_6H_2I_2$-O-$C_6\underline{H}_2I_2$-CH_2-), 7.50 (m, 1H, *p*-C_6H_5-CS_2-), 7.34 (m, 2H, *m*-C_6H_5-CS_2-), 7.10 (m, 2H, HO-$C_6\underline{H}_2I_2$-O-$C_6H_2I_2$-CH_2-), 4.08 (bs, C(O)OC$\underline{H}_2$-), 3.66, 3.61, 3.54 (3 bs, C(O)O-$CH_2C\underline{H}_2$-O-$C\underline{H}_2C\underline{H}_2$-O-$CH_3$), 3.37 (bs, -O-C$\underline{H}_3$), 2.96 (m, 2H, -$C_6H_2I_2$-C$\underline{H}_2$-), 2.36 (m, 2H, C$\underline{H}_2$-C(O)NH-), 2.0−1.2 (m, -C$\underline{H}_2$-C(CH_3)(COO-)), 1.4−0.7 (m, -CH_2-C(C$\underline{H}_3$)(COO-)); ^{13}C NMR ($CDCl_3$, 100 MHz, δ/ppm) 177.2 (-CH_2-C(CH_3)(C$\underline{O}$O-)), 141.1 (Ar-O-C-C(I)-$\underline{C}$H-), 132.4 (*p*-C_6H_5-CS_2-), 128.3 (*m*-C_6H_5-CS_2-), 126.7 (*o*-C_6H_5-CS_2-), 126.0 (HO-C-C(I)-$\underline{C}$H-), 71.9, 70.4, 68.6 (C(O)O-$CH_2\underline{C}H_2$-O-$\underline{C}H_2\underline{C}H_2$-O-$CH_3$), 63.9 (C(O)O-$\underline{C}H_2$-), 59.0 (-O-$\underline{C}H_3$), 54.4 (-$\underline{C}H_2$-C($CH_3$)(COO-)), 45.0, 44.6 (-$CH_2$-$\underline{C}$($CH_3$)(C$\underline{O}$O-)), 41.0 (-$C_6H_2I_2$-$\underline{C}H_2$-), 31.9 ($\underline{C}H_2$-C(O)-NH-), 16.6, 18.6 ($CH_2$-C($\underline{C}H_3$)(COO-)).

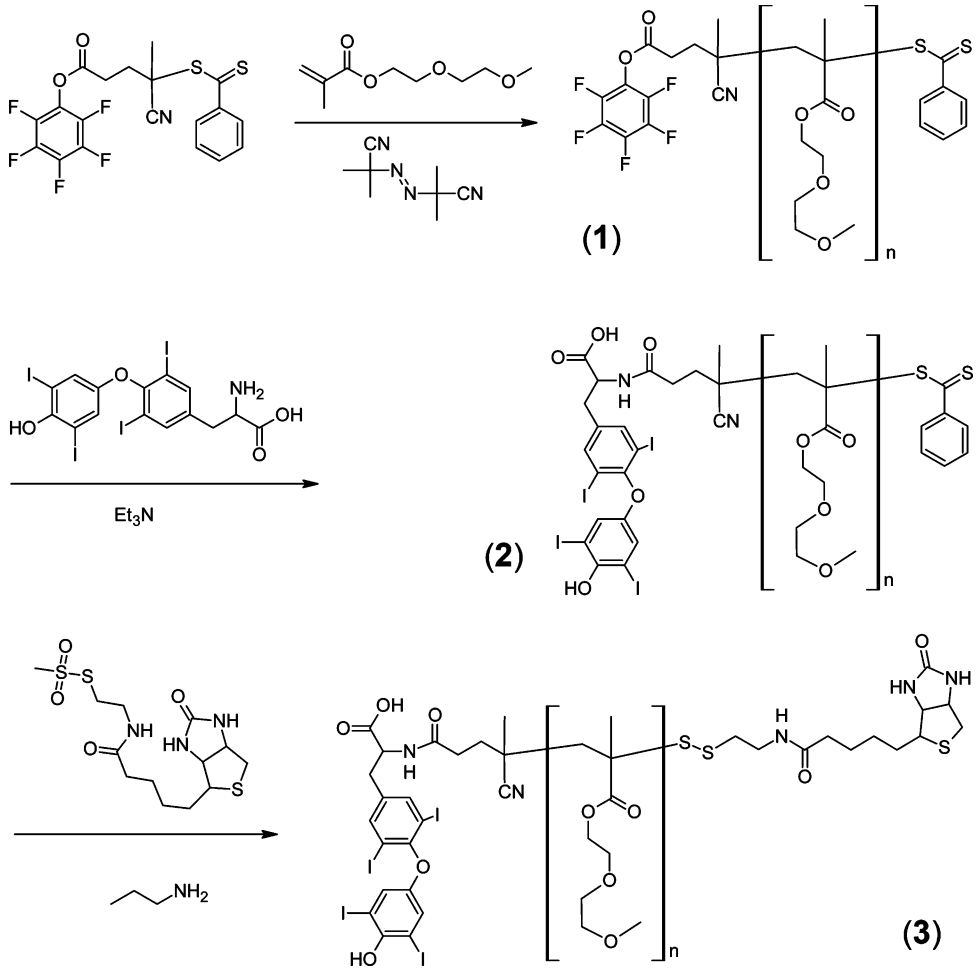
α-Thyroxin, ω-Biotin Poly[diethyleneglycol monomethylether methacrylate] (T4-PDEGMA-biotin, 3). To 850 μL of a 6.9 mM solution of T4-PDEGMA-DTE in dry DMSO (5.87 μmol), 30 mg (78.6 μmol) of *N*-biotinylaminoethyl methanethiosulfonate was added, and after 1 min, 30.2 μL (367 μmol) of *n*-propyl amine was added. The mixture was stirred overnight at room temperature. The product was purified by dialysis in methanol for 2 days, with solvent changes three times a day. T4-PDEGMA-biotin was obtained in 32% yield. M_n (GPC, PS-equivalent) = 6.3 kg /mol; PDI (GPC) = 1.14; M_n (UV−vis) = 7030 kg/mol. ^{1}H NMR ($CDCl_3$, 400 MHz, δ/ppm) 7.72 (m, 2H, HO-$C_6H_2I_2$-O-$C_6\underline{H}_2I_2$-CH_2-), 7.13 (m, 2H, HO-$C_6\underline{H}_2I_2$-O-$C_6H_2I_2$-CH_2-), 4.55 (m, 2H, NHC$\underline{H}$CH(CH_2)-S-$CH_2C\underline{H}$NH-), 4.35 (m, NHC$\underline{H}$CH(CH_2)-S-CH_2CHNH-), 4.08 (bs, C(O)OC$\underline{H}_2$-), 3.66, 3.61, 3.54 (3 bs, C(O)O-$CH_2C\underline{H}_2$-O-$C\underline{H}_2C\underline{H}_2$-O-$CH_3$), 3.37 (bs, -O-C$\underline{H}_3$), 3.18 (m, 2H, NHCH-CH($CH_2$)-S-C$\underline{H}_2$CHNH-), 2.93 (m, 2H, -$C_6H_2I_2$-C$\underline{H}_2$-), 2.76 (m, 2H, -SS-C$\underline{H}_2$-), 2.39 (m, 2H, C$\underline{H}_2$C(O)NH-C(COOH)), 2.22 (m, C$\underline{H}_2$C(O)NH-CH_2CH_2SS), 2.0−1.2 (m, -C$\underline{H}_2$-C(CH_3)(COO-)), 1.4−0.7 (m, -CH_2-C(C$\underline{H}_3$)(COO-)); ^{13}C NMR ($CDCl_3$, 100 MHz, δ/ppm) 177.3 (-CH_2-C(CH_3)(C$\underline{O}$O-)), 141.2 (Ar-O-C-C(I)-$\underline{C}$H-), 126.0 (HO-C-C(I)-$\underline{C}$H-), 71.9, 70.5, 68.5 (C(O)O-$CH_2\underline{C}H_2$-O-$\underline{C}H_2\underline{C}H_2$-O-$CH_3$), 63.9 (C(O)O-$\underline{C}H_2$-), 61.5 (NH$\underline{C}$HCH($CH_2$)-S-$CH_2$CHNH-), 60.0 (NHCHCH($CH_2$)-S-$\underline{C}H_2$CHNH-), 59.1 (-O-$\underline{C}H_3$), 54.4 (-$\underline{C}H_2$-C($CH_3$)(COO-)), 45.1, 44.7 (-$CH_2$-$\underline{C}$($CH_3$)(C$\underline{O}$O-)), 41.0 (-$C_6H_2I_2$-$\underline{C}H_2$-), 37.6 (SS-$\underline{C}H_2$), 31.8 ($\underline{C}H_2$-C(O)NH-), 16.7, 18.5 ($CH_2$-C($\underline{C}H_3$)(COO-)).

Bis(5-carboxypentyl) disulfide bis thyroxin amide, **4**,[59] and bis(6-hydroxyhexyl) disulfide, **5**,[60] were prepared as previously described.

Results and Discussion

RAFT polymerization was employed as a method to produce a polymer featuring biotin and thyroxin (T4) end groups. These

Scheme 1. Reversible Addition−Fragmentation Chain Transfer (RAFT) Polymerization of Diethylene Glycol Monomethylether Methacrylate Using a Dithioester Chain Transfer Agent Carrying an Activated Pentafluorophenyl Ester and Successive Functionalization of the End Groups with the Thyroid Hormone Thyroxin and Biotin



biomolecules were chosen as models because each of them specifically targets a protein. The strong specific binding of biotin to streptavidin has extensively been investigated and exploited.[26–28] Thyroxin binds to its three transport proteins: thyroxin binding globulin (TBG), human serum albumin, and prealbumin (transthyretin), which are responsible for distributing the hormone to target tissues.[57,58] Prealbumin recognizes phenols, which are halogenated in both ortho positions, and thus also binds to thyroxin if the amino group on the opposite side has been functionalized.[58,59]

A dithioester (DTE) chain transfer agent (CTA) carrying a pentafluorophenyl (PFP) activated ester was used for polymerization of diethyleneglycol monomethyl ether methacrylate (DEGMA; Scheme 1).

The resulting polymer **1** had a polystyrene-equivalent molecular weight of 8 kg/mol with a narrow molecular weight distribution (PDI = 1.14), as determined by gel permeation chromatography (GPC) measurement (Figure 1).

To exactly calculate the stoichiometric amounts for the further reactions, an absolute molecular weight of M_n = 6250 g/mol was calculated from the absorbance of the retained dithioester end group using a calibration curve.[56] To further verify this value, the weight-average molecular weight of polymer **1** was determined by static light scattering to be M_w = 6900 g/mol, which was in good agreement with the value obtained by UV−vis spectroscopy, when the polydispersity index M_w/M_n is taken into account. The PFP α end group was clearly visible in a ^{19}F NMR measurement and IR spectroscopy revealed the characteristic carbonyl band at 1778 cm^{-1} and the aromatic C=C valence band at 1520 cm^{-1} (see Supporting Information). The aromatic signals of the phenyl dithioester ω end group could be observed in ^{1}H NMR spectroscopy (Figure 2) and the characteristic absorbance of the dithioester centered at 302 nm, recorded by a UV−vis measurement, is shown in Figure 3.

The biofunctionalization of PFP-PDEGMA-DTE **1** proceeded in two steps. Both end groups were reactive toward amines. However, as the PFP esters show a higher reactivity toward amines (see additional experiments described in Supporting Information),[44,61] the PFP α end group could selectively be

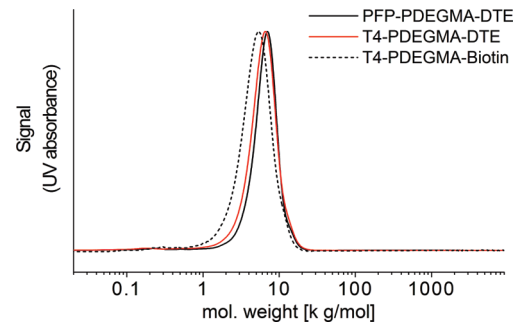


Figure 1. Gel permeation chromatograms of PFP-PDEGMA-DTE **1** (black curve), T4-PDEGMA-DTE **2** (red curve), and T4-PDEGMA-biotin **3** (dotted curve).

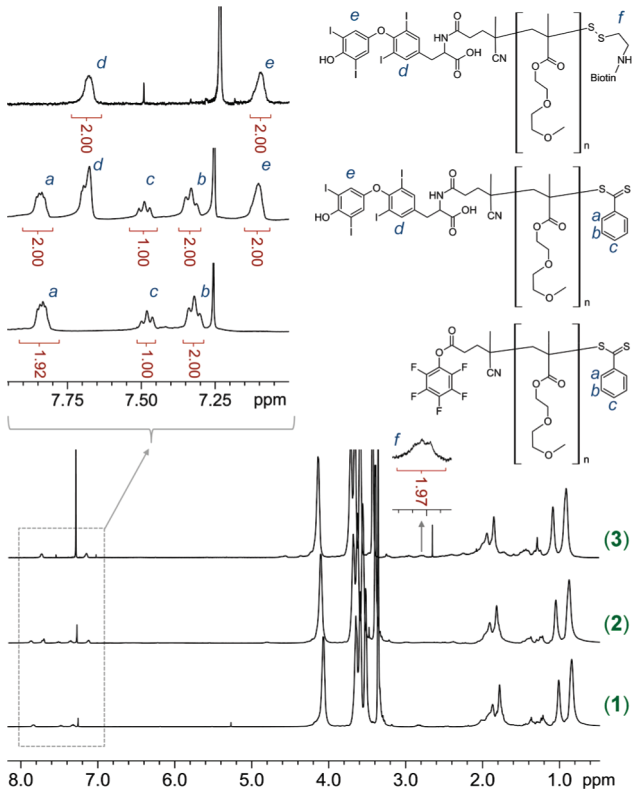


Figure 2. NMR spectra of PFP-PDEGMA-DTE **1** (bottom), T4-PDEGMA-DTE **2** (middle), and T4-PDEGMA-biotin **3** (top) and sections showing the retention of the ω-terminal dithioester upon introduction of the α-thyroxin group and the complete removal of the dithioester upon substitution with the biotin terminus.

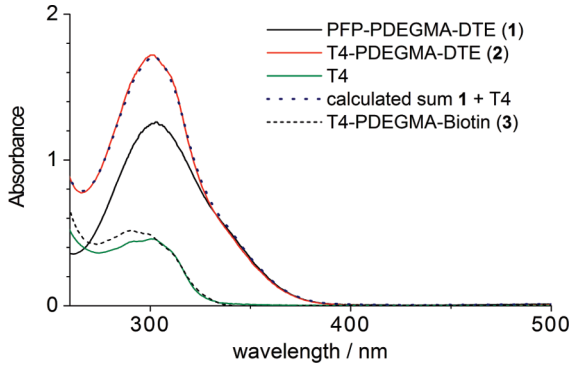


Figure 3. UV−vis absorbance spectra of PFP-PDEGMA-DTE **1** showing the absorbance of its terminal dithioester (black curve), of thyroxin (green curve), of T4-PDEGMA-DTE **2**, giving rise to a spectrum superimposed of dithioester absorbance and thyroxin absorbance (red curve), of a calculated linear combination of thyroxin absorbance and dithioester absorbance (blue dotted curve), nicely matching the measured spectrum of T4-PDEGMA-DTE **2**, and of the product T4-PDEGMA-biotin **3** (black dashed line). All curves were measured at (or normalized to) a concentration of 150 μmol/L.

reacted with the amino group of 1 equiv of thyroxin (Scheme 1). The complete conversion of the PFP ester at the α end group was verified by the absence of fluorine signals in ^{19}F NMR measurement (see Supporting Information), while UV−vis showed that the ω-terminal DTE remained quantitatively (Figure 3). GPC showed that the molecular weight and the PDI of T4-PDEGMA-DTE **2** had not changed from the starting polymer. ^{1}H NMR spectroscopy showed the two distinct aromatic signals of the new thyroxin end group. Additionally, the aromatic signals of the DTE were still present, confirming that the ω end group had not been harmed. The conversion of the PFP ester with T4 as well as the preservation of the DTE were both estimated to be quantitative though integration of the signals. However, an error of 5−10% should be allowed for this method. Higher precision evidence could be gained from a UV−vis absorbance measurement. The UV−vis spectrum of T4-PDEGMA-DTE **2** consisted of two superimposed peaks originating from the ω-DTE and the α-T4 group (Figure 3). A linear combination of the expected absorbencies matched the measured curve, suggesting a quantitative presence of both end groups (dotted line in Figure 3). These conversions were consistent with the values from NMR integration and showed that a selective modification of a PFP ester is possible in the presence of a dithioester. This approach thus allowed the further modification of the ω DTE by means of aminolysis in the presence of a biotinylated methane thiosulfonate (MTS, see Scheme 1). The method of employing functional MTS reagents has been shown to quantitatively introduce functional ω disulfides groups during aminolysis.[54,55] Disulfides are very stable in the absence of reducing agents or radicals and are often employed as linkers between polymers and biomolecules.[15,16,30,36,40,45,62] After reaction of T4-PDEGMA-DTE **2** with biotin-MTS (see Scheme 1), the DTE absorbance peak had disappeared from the UV−vis absorbance spectrum, with only the absorbance of the T4 α end group remaining (Figure 3). The aromatic signals of the phenyl dithioester group had also completely disappeared from the ^{1}H NMR spectrum (Figure 2). These measurements indicated a complete conversion of the DTE end group. At 4.55 and 4.35 ppm, the typical biotin CH signals appeared. However, due to overlapping with other signals, the integration values of these peaks were too high. For integration, the signal of the methylene group adjacent to the disulfide bridge at 2.76 ppm (^{13}C signal at 37.6 ppm seen in an HSQC spectrum) was used, yielding a value of 1.97, suggesting a 98.5% presence of the two expected protons. Even with granting this method an error of several percent, it still accounts for an almost quantitative conversion also on the ω end group of the polymer. The obtained heterotelechelic biofunctionalized polymer **3** was soluble in THF, DMF, chloroform, and in water at room temperature, therewith demonstrating the influence of the polymer in overcoming solubility issues of the T4 group in organic solvents. A gel permeation chromatogram of T4-PDEGMA-biotin **3** showed a monomodal curve of the same width as the precursor polymers **1** and **2**, however, it was shifted toward a lower molecular weight. This behavior had already been observed in other cases where a dithioester had been removed from a polymer and was attributed to a denser coiling of the polymer around its new end group, that is, a slightly higher degree of swelling of the polymer coil having a dithioester end group (Figure 1).[54,55] Noteworthy, no peak nor shoulder of double molecular weight had appeared, indicating that no side reactions such as formation of polymer−polymer disulfides (which are favored in the absence of MTS reagents) had occurred and that the end group exchange had proceeded quantitatively as intended. The presence of the terminal biotin moiety could further be confirmed by IR spectroscopy. The spectra of the starting polymer PFP-PDEGMA-DTE **1** and of the product T4-PDEGMA-biotin **3** were compared. The characteristic bands of the PFP end group at 1778 and 1520 cm^{-1} were absent from the IR spectrum of polymer **3**. In contrary, the urea-carbonyl absorption of biotin around 1680 cm^{-1} and the C−N stretch absorption at 1259 cm^{-1} of biotin appeared in the spectrum of T4-PDEGMA-biotin (see Supporting Information). In summary,

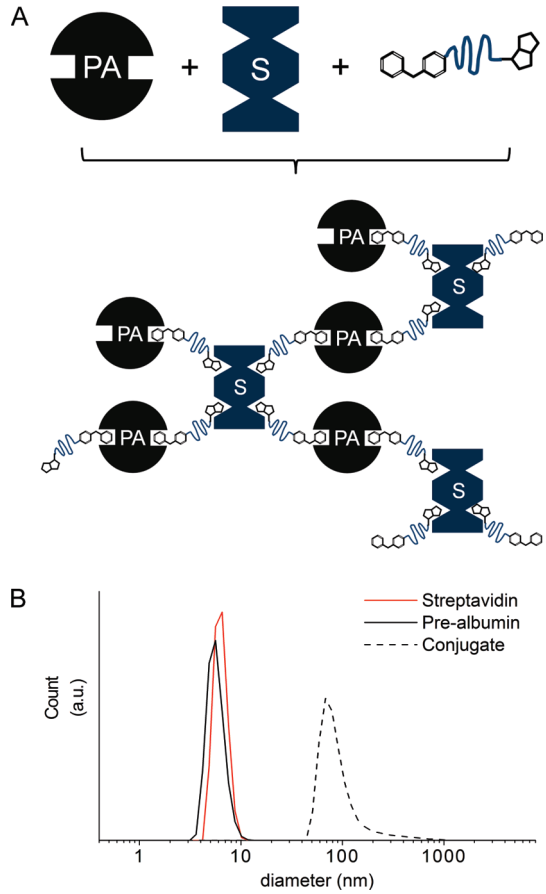


Figure 4. (A) Schematics showing the formation of a 3-dimensional network of prealbumin (P; with two binding sites for thyroxin), streptavidin (SA; with four binding sites for biotin), and the heterotelechelic polymer T4-PDEGMA-biotin **3**. (B) Results of dynamic light scattering on the individual proteins streptavidin (red curve) and prealbumin (back curve) and of the polyconjugate formed with polymer **3** (dashed line).

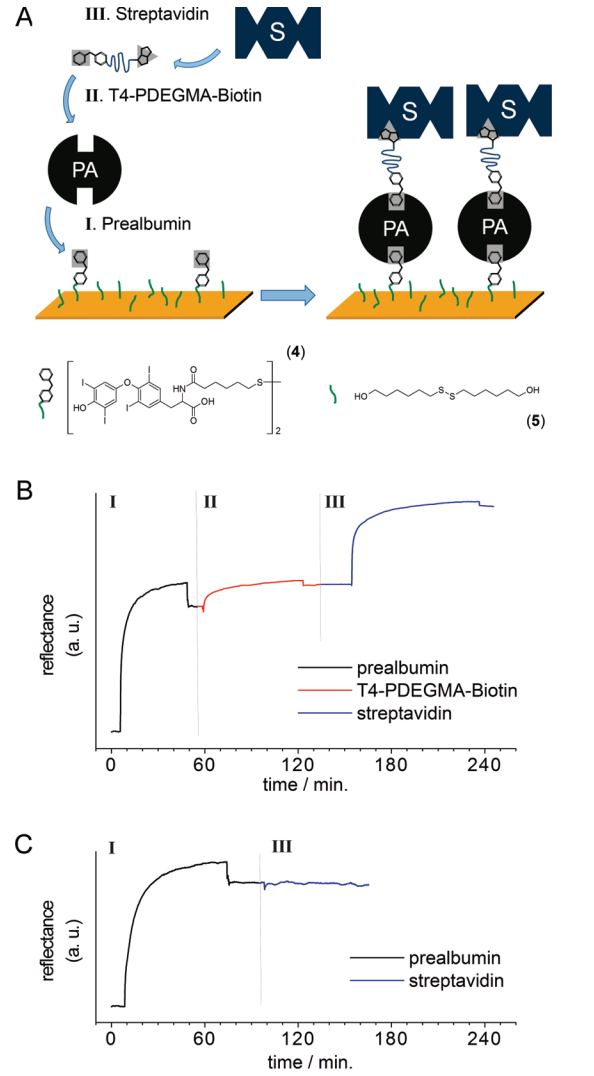


Figure 5. (A) Schematics showing the successive immobilization of prealbumin, T4-PDEGMA-biotin **3** and streptavidin onto a self-assembled monolayer of T4-disulfide **4** and hydroxyl-disulfide **5** on a planar gold surface. (B) Surface plasmon resonance (SPR) measurement showing the increase of optical thickness upon immobilization of prealbumin (black curve), T4-PDEGMA-biotin **3** (red curve), and streptavidin (blue curve). (C) SPR measurement of the binding of prealbumin onto a self-assembled monolayer of disulfides **4** and **5** (black curve) and attempted immobilization of streptavidin (blue curve).

these data confirmed the successful α and ω biofunctionalization of the precursor polymer **1** in a two-step procedure with very high yields.

The bioconjugation of both end groups of polymer **3** was first investigated in solution. Diluted buffered solutions of prealbumin, streptavidin and T4-PDEGMA-biotin **3** were combined. As prealbumin features two thyroxin binding sites and streptavidin exhibits four biotin binding sites, a molar ratio of 1 equiv of streptavidin/2 equiv of prealbumin/2 equiv of polymer **3** was chosen to aim at a high conjugation, resulting in a three-dimensional super structure (Figure 4A).

Such an adduct can only be formed if polymeric connectors exhibiting both a biotin and a thyroxin end group are present. Dynamic light scattering was measured of each individual protein and of the conjugate with polymer **3** (Figure 4B). As expected, both streptavidin and prealbumin gave rise to monomodal narrow peaks at 7 and 5.8 nm hydrodynamic diameter, respectively. The polymer had a hydrodynamic diameter of 3.8 nm (not shown). After addition of polymer **3** to a mixture of both proteins, the signals corresponding to the individual proteins had vanished and were replaced with a broad peak around 70 nm hydrodynamic diameter with a shoulder extending toward larger diameters. This measurement confirmed the formation of a protein/polymer microgel, which had consumed both individual protein building blocks, thus demonstrating the heterotelechelic nature of polymer **3**. It also showed the successful protein identification of the polymer end groups. Such 3-dimensional superstructures consisting of protein and polymer components are gaining an increasing importance in the development of biomaterials and in research field of tissue engineering.[63,64]

Next, the polymer with its two different biotarget end groups was used in a directed surface immobilization, which was monitored by surface plasmon resonance (SPR). Disulfide **4** carrying T4 groups was used for biolabeling of a planar gold surface (Figure 5A).

To reduce any nonspecific binding of proteins to a nonpolar T4 surface, a mixture of disulfide **4** and bis(6-hydroxyhexyl)-disulfide **5** (molar ratio 1:12) was self-assembled onto the gold surface.[30,65] After thorough rinsing, prealbumin in PBS buffer was injected and a rapid binding of the protein to the surface was observed (Figure 5B, part I). The average thickness of this first protein layer was fitted to be 14.4 Å, which was somewhat lower than 26.0 Å found for a dense prealbumin monolayer[59]

and, therefore, was in agreement with the T4 targets being "diluted" with hydroxyl groups on the surface.[65] After rinsing the prealbumin coated surface with PBS buffer, T4-PDEGMA-biotin **3** was injected into the cell. As each prealbumin has two binding sites for a T4 molecule but does not interact with biotin or the polymer spacer, the observed thickness increase of 1.9 Å indicated the attachment of the T4 end groups of polymer **3** onto the prealbumin coated surface (Figure 5B, part II). Next, streptavidin was injected, which bound rapidly to the surface already containing a prealbumin and a polymer layer, resulting in a thickness increase of 23.4 Å (Figure 5B, part III). This showed that biotin groups had been expressed on the surface and were available for streptavidin conjugation. In dense monolayers, streptavidin produces layers with a thickness of around 52 Å.[59] The lower value found here thus proposed that only binding to the biotin end groups had taken place and no nonspecific binding had occurred.

As SPR measures an average thickness, a 55.4% surface coverage with prealbumin was estimated from the ratio of thickness measured here and that of a dense monolayer. Streptavidin built up a layer with an average thickness of 45% that of a dense monolayer. From these values, an immobilization efficiency of 81% based on prealbumin was estimated, suggesting an accurate site-specific conjugation of streptavidin onto the biotin end groups. The same experiment was repeated, and this time streptavidin was directly injected onto the prealbumin coated surface, that is, polymer **3** was omitted. In this case, no attachment of streptavidin to the surface and thus no increase in reflectance were found, proving that (i) polymer **3** was essential for the recognition and conjugation of the two different proteins via its end groups (Figure 5C) and that (ii) nonspecific binding of streptavidin could be excluded.

Conclusion

The synthesis of a polymer carrying two different terminal biofunctionalities was described. The synthesis featured the use of a chain transfer agent featuring a pentafluorophenyl ester, which was retained as α end group of the polymer. Due to its high reactivity toward biological amines, α amidation was possible while leaving the dithioester intact. The dithioester *ω* end group could then be subjected to an aminolysis in the presence of a functional methane thiosulfonate (MTS) reagent, which is a powerful method to introduce terminal functionalities with very high yields. The combination of α-PFP esters with functional MTS reagents thus presents a versatile pathway toward heterotelechelic (bio)functionalized polymers. As both steps of the synthesis can be performed at room temperature, there is no risk of decomposition of ester groups and thus, poly[(meth)acrylates] are eligible for this method. In the present case, α-thyroxin and *ω*-biotin end groups were introduced into a poly[diethylene glycol monomethylether methacrylate]. These end groups were specifically targeted by prealbumin and streptavidin, respectively, and a subsequent immobilization onto a surface was possible with the polymer connecting the two different proteins with its end groups. Combination of the building blocks in solution resulted in the formation of a 3-dimensional super structure that could be observed in dynamic light scattering. Overall, the polymer presented here has the potential of acting as a molecular adapter for two different proteins, such as prealbumin to streptavidin and vice versa, which could then offer the broad range of analysis and conjugation possibilities available for streptavidin surfaces. Additionally, as a stimulus responsive polymer, poly[diethyleneglycol monomethylether methacrylate] invites to explore possibilities of reversible or stimulus-controlled conjugation.

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Supporting Information Available. IR spectra of polymers **1** and **3**; ^{19}F NMR spectra of polymers **1** and **2**, data illustrating the reactivity difference of pentafluorophenyl esters versus dithioester toward amines. This material is available free of charge via the Internet at http://pubs.acs.org.

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