Polym. Bull. (2012) 68:681–691
DOI 10.1007/s00289-011-0570-4

ORIGINAL PAPER

Synthesis of poly(ethylene glycol)-*b*-poly(mercapto ethylacrylamide) diblock copolymer via atom transfer radical polymerization

Aleya Hasneen · In-sung Cho · Keon-Woo Kim · Hyun-jong Paik

Received: 7 June 2010 / Accepted: 29 June 2011 / Published online: 26 July 2011

Abstract Atom transfer radical polymerization was used to synthesize a well-defined poly(ethylene glycol)-*b*-poly(mercapto ethylacrylamide) (PEG-*b*-PMEAAm) diblock copolymer. Poly(ethylene glycol)-*b*-poly[*N*-(acryloxysuccinimide)](PEG-*b*-PNAS) was synthesized at 80 °C using methoxy-poly(ethylene glycol)-2-bromo propanoate (PEG-Br) and CuBr/2,2′-bipyridine as a macroinitiator and catalyst, respectively. The monomer conversion was determined by ^{1}H nuclear magnetic resonance (NMR) spectroscopy. The resulting PEG-*b*-PNAS diblock copolymer was characterized by gel permeation chromatography, Fourier transform infrared (FT-IR), and ^{1}H NMR spectroscopy. Disulfide groups were introduced by a simple reaction through the *N*-acryloxysuccinimide (NAS) moieties of the PEG-*b*-PNAS diblock copolymer with cystamine dihydrochloride in the presence of triethylamine. FT-IR spectroscopy was used to confirm the introduction of disulfide moieties into the polymer repeating units. Subsequently, a thiol-functionalized block copolymer was prepared using DL-dithiothreitol (DTT) as the reducing agent and the reduction step was monitored by ^{1}H NMR spectroscopy. This thiol group was transformed easily to a disulfide bond using $FeCl_3$ as an oxidizing agent. The transformation into disulfide could be visualized easily as insoluble polymeric particles formed from a clear solution of PEG-*b*-PMEAAm after oxidation.

Keywords Atom transfer radical polymerization (ATRP) · Block copolymer · Poly(ethylene glycol)-*b*-poly(mercapto ethylacrylamide) · Thiol

Introduction

Thiol is one of the most distinctive functional groups that is used extensively in many areas of chemistry. Thiol plays a crucial role in protein folding, signal

A. Hasneen · I. Cho · K.-W. Kim · H. Paik (✉)
Department of Polymer Science and Engineering, Pusan National University, Busan 609-735, Korea
e-mail: hpaik@pusan.ac.kr

transduction, cell proliferation, and various redox processes because of its unique ability for reversible thiol–disulfide bond formation [1]. Moreover, the specific interaction of the thiol group with metals, such as gold, silver, and cadmium has led to many applications in materials science, including optical, electronic, magnetic, catalytic, and biomedical fields [2–4]. Recent advances in the areas of biochemistry and microelectronics have further increased the need for a straightforward synthetic approach to thiol-functional materials [5–7].

In particular, thiol-containing polymers have been the subject of extensive research and are used widely in the derivatization of interfaces with synthetic and biological molecules in tissue engineering, protein arrays, molecular diagnostics, and drug delivery [8–15]. For example, thiol-end functionalized poly(ethylene)glycol-block-poly(DL-lactic acid) (PEG-*b*-PDLLA) synthesized by modifying the amine end group by *N*-succinimidyl 3-maleimidopropionate has been investigated for the preparation of biomimetic surfaces [9]. Although, there are reports available on thiol-containing polymers, only a few have reported the synthesis of thiol-functionalized block copolymers [16, 17]. In this context, there has been particular interest in determining a more efficient synthetic method for well-defined thiol-containing copolymers.

To date, a wide variety of thiol-containing polymers have been prepared, in which the thiol group is mostly present at the chain end. Reversible addition-fragmentation chain transfer polymerization (RAFT) is one of the most widely used methods for synthesizing thiol-end functionalized polymers. The introduction of thiol-functionality into a polymer using a RAFT technique has been performed mainly using protection–deprotection approach, which occurs via the reduction of disulfide groups under basic conditions [18–22].

Atom transfer radical polymerization (ATRP) [23] is also used to prepare thiol-containing polymers [24–26]. Thiol-functionalized polymers can be prepared by ATRP in two ways. One is a reaction between the halogen end groups and sulfur-containing nucleophilic precursor of the thiol group e.g., (hydrogen) sulfide salts, thiourea [27, 28], thiodimethylformamide [29, 30] etc. Another way is employing the concept of mono or polyfunctional halide initiators containing protected thiol group. For example, methyl methacrylate has been polymerized by ATRP using a 2,4-dinitrophenyl-protected thiol-containing initiator [31]. The use of such a thiol precursor in ATRP is limited due to the significantly lower transfer constants of the thioethers compared to those of thiols. However, there are no reports of polymers with pendant thiol-functionality at each repeating unit by ATRP.

This article reports an easy method to synthesize a poly [(ethylene glycol)-*b*-poly (mercapto ethylacrylamide)] diblock copolymer with pendant thiol groups by ATRP. The synthesized block copolymers have potential in many biological applications owing to its hydrophilic and biocompatible poly(ethylene)glycol block and poly(ethylacrylamide) block, which has a thiol group in each repeating unit. In addition, using the reversible formation of thiol/disulfide bond in the pendant group, crosslink of block copolymers can be controlled by the redox reactions.

Experimental

Materials

N-hydroxysuccinimide (NHS, 98%), poly(ethylene glycol)methyl ether (PEG-OH, $M_n = 2000$ g/mol), 2-bromopropionyl bromide (BPB, 97%), 2,2′-bipyridine (bpy, 99%), cystamine dihydrochloride (98%), DL-dithiothreitol (DTT, 99%), and iron(III) chloride ($FeCl_3$, 97%) were purchased from Aldrich and used as received. Copper (I) bromide (Aldrich, 98%) was purified according to the literature procedure [32]. Triethylamine (Junsei, 99%) and acryloyl chloride (TCI, stabilized with MEHQ) were used as received. All other solvents were of reagent grade and used directly without further purification.

Characterization

The molecular weights (M_n) and molecular weight distributions (M_w/M_n) were determined by gel permeation chromatography (GPC) in 0.1% LiBr-containing DMF as the eluent. GPC was calibrated with a poly(methyl methacrylate) standard and equipped with a Waters 515 pump, Waters 2414 RID detector, and HSPgel HR MB-L (6.0 × 150 mm) column. The ^{1}H nuclear magnetic resonance (NMR) spectra were obtained on a Varian Unity Plus 300 spectrometer. The Fourier transform infrared (FT-IR) spectra were recorded on a Thermo Scientific Nicolet 6700 FT-IR spectrometer. Dynamic laser scatterings (DLS) measurement was performed using a Brookhaven 90Plus Nanoparticle Size Analyzer.

Synthesis of NAS

NAS was synthesized using a modification of the procedure reported elsewhere [33]. Acryloyl chloride (8.50 mL, 1.2 eq.) was added dropwise to a stirred solution of *N*-hydroxysuccimide (10.0 g, 86.9 mmol) and TEA (14.6 mL, 1.2 eq.) in chloroform (150 mL) at 0 °C. After 4 h, the solution was extracted with deionized water and the organic phase was dried over $MgSO_4$. The solution was concentrated and the crude product was recrystallized from hexane/ethyl acetate (8:1). The crystals were filtered and washed successively with 60 mL of hexane/ethyl acetate (4:1), 60 mL of hexane/ethyl acetate (9:1), and 60 mL of hexane, and then dried to give a white solid in 77% yield. ^{1}H NMR (300 MHz, DMSO-d_6), $\delta = 6.32$–6.70 (m, 3H, CH_2=CH), 2.84 (s, 4H, CH_2CH_2).

Synthesis of PEG-Br

PEG-Br was synthesized using a modification of the procedure reported elsewhere [34]. 2-bromopropionyl bromide (0.80 mL, 7.50 mmol) was added dropwise to a solution of PEG (3.00 g, 1.50 mmol) and TEA (1.05 mL, 7.50 mmol) in THF (75 mL) at 0 °C. The solution was then stirred for 12 h at room temperature. The solvent was evaporated, and the residue was dissolved in chloroform (30 mL) and extracted with deionized water. After the evaporation of chloroform, the

concentrated solution was precipitated into cold diethyl ether. The product was dried under vacuum. (71% yield) ^{1}HNMR (300 MHz, $CDCl_3$), $\delta = 1.76$ (d, 3H, Br**C**$\mathbf{H}_3$CHCOO–), 3.31 (s, 3H, CH_3O–), 3.40–3.73 (m, 178H, [–CH_2CH_2O–]–), 4.25 (m, 1H, –CH–COO–), 4.34 (m, 2H, –BrCH$_3$CHCOOC$\mathbf{H}_2$–).

Synthesis of PEG-*b*-PNAS block copolymer

NAS (1.50 g, 8.87 mmol), PEG-Br (381 mg, 0.180 mmol), CuBr (25.4 mg, 0.180 mmol), and bpy (55.4 mg, 0.36 mmol) were added to a N_2 purged Schlenk flask and sealed immediately with a rubber septum. The Schlenk flask was degassed with three freeze–pump–thaw cycles and filled with nitrogen. The Schlenk flask was then immersed in an oil bath at a preset temperature of 80 °C. After 3 h, a small amount of sample was taken and dissolved in deuteriated DMSO for ^{1}H NMR analysis to determine the monomer conversion. The remaining reaction mixture was dissolved in DMSO and precipitated in a large volume of methanol. The solid product obtained was washed several times with methanol, dried under vacuum, and analyzed by ^{1}H NMR, GPC, and FT-IR.

Synthesis of PEG-*b*-PAAm(-*S*-*S*-)

The PEG-*b*-PNAS diblock copolymer (0.50 g, 2.30 mmol) was dissolved in 10 mL of distilled DMF. An aqueous solution of cystamine dihydrochloride (0.86 g in 2.0 mL water, 4.60 mmol) and TEA (2.50 mL, 18.40 mmol) were added to the solution at room temperature, and stirred constantly for 12 h. After this period, the solvent was evaporated and the residue was dialyzed against deionized water for 3 days. A fine powder was obtained by freeze-drying. The structure of the obtained polymer was characterized by FT-IR spectroscopy.

Reduction of PEG-*b*-PAAm (-*S*-*S*) to PEG-*b*-PMEAAm

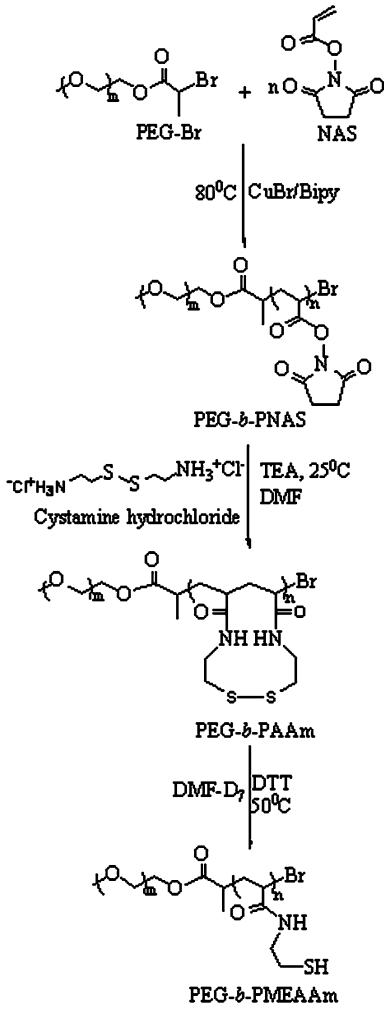
The PEG-*b*-PAAm cross-linked polymer (5.0 mg, 0.09 mmol) was dispersed in 2 mL of degassed DMF-d_7, followed by the addition of dithiothreitol (27.76 mg, 0.90 mmol). After heating at 50 °C for 17 h, the opaque suspension turned into a transparent solution. The transformation was monitored at different time intervals by ^{1}H NMR spectroscopy.

A half-portion of the solution was removed from the same reduction reaction and diluted with DMF. $FeCl_3$ (0.60 mmol, 0.096 mg) was then added as an oxidized agent and heated to 60 °C with constant stirring. Insoluble particles were observed at the bottom of the vial due to oxidation after 12 h.

Results and discussion

Scheme 1 shows the synthesis of the thiol-functionalized poly(ethylene glycol)-*b*-poly(mercapto ethylacrylamide diblock copolymer. Direct polymerization of a thiol-containing monomer is a not feasible pendant unit to the polymeric backbone

Scheme 1 Synthesis of poly(ethylene glycol)-*b*-poly(mercapto ethylacrylamide)



due to spontaneous crosslinking of the thiol group to a disulfide group. Therefore, a preactivated ester monomer, NAS, was used to ensure pendant thiol groups at each repeating unit through cross-linking with cystamine in the presence of triethylamine followed by reduction of the disulfide bond in the cross-linked polymer.

The PEG-*b*-PNAS block copolymer was synthesized by ATRP using PEG-Br and CuBr/bpy as the macroinitiator and catalyst, respectively. Polymerization proceeded well in the bulk at 80 °C, which is higher than the melting point of NAS (69 °C) [35]. The monomer conversion was determined by comparing the integration values of the methylene protons (I_b) in the PNAS unit and vinyl protons from monomer (I_a) PNAS in ^{1}H NMR spectrum (data not shown). The calculated conversion was 68%.

The resulting crude polymer was then dissolved in DMSO and purified by precipitation in a large amount of MeOH. The molecular weight of the PEG-*b*-PNAS block copolymer was determined by GPC analysis (Fig. 1). The GPC

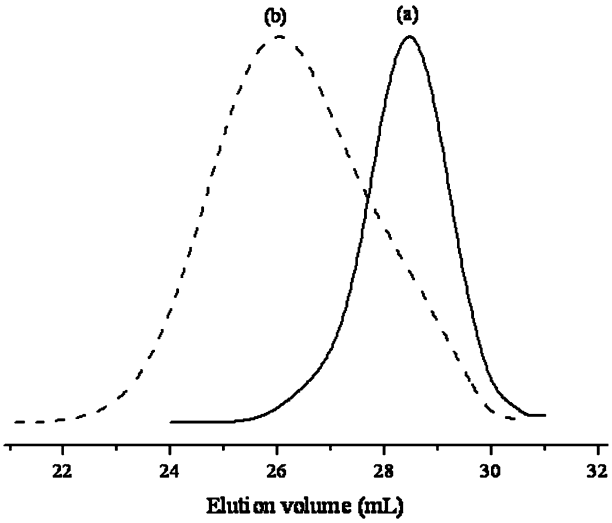


Fig. 1 GPC chromatograms of *a* PEG-Br macroinitiator and *b* PEG-*b*-PNAS diblock copolymer

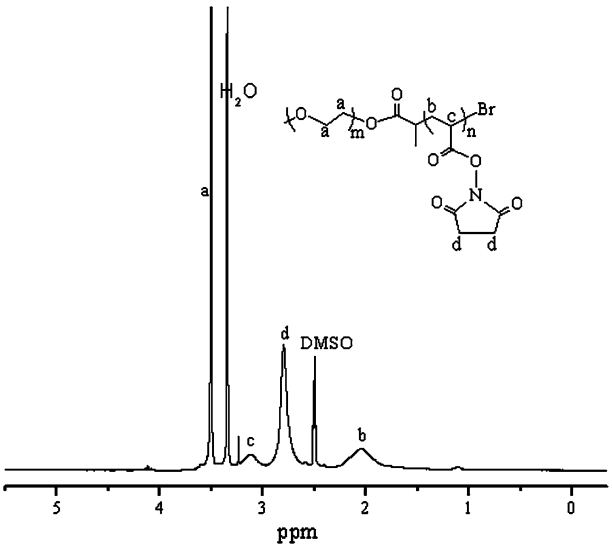


Fig. 2 ^{1}H NMR spectrum of PEG-*b*-PNAS in DMSO-d_6 solvent

molecular weight was in good agreement with the theoretical value based on monomer conversion and the value obtained from ^{1}H NMR spectroscopy (see Table 1).

The molecular weight was determined from the ^{1}H NMR data using the following equation, and by taking the ratio of the integrated values corresponding to the methine protons (I_c) of PNAS repeating unit and methylene protons (I_a) from the PEG moiety (Fig. 2). FT-IR spectroscopy was used to characterize the

Table 1 Characterization of poly(ethylene glycol)-*b*-poly (*N*-acryloxy succinimide) block copolymer prepared by ATRP at 80 °C

Entry no.	Polymer	Conversion (%)	M.W. (g/mol)[a] theoretical	M.W. (g/mol)[b] NMR	M.W. (g/mol)[c] GPC	M_w/M_n
1	PEG-*b*-PNAS	68.05	7890	9750	9650	1.33

1 $[NAS]_0:[I]_0:[CuBr]_0:[Bipy]_0 = 50:1:1:2$

[a] $([M]_0/[I]_0 \times$ conversion $\times$ M.W. of monomer) + M.W. of initiator

[b] Determined by ^{1}H NMR using the equation $(180 \times I_c/I_a \times 169 + 2150)$ g/mol

[c] Determined by GPC using 0.01 M LiBr containing DMF as eluant calibrated with poly(methyl methacrylate) standards

PEG-*b*-PNAS block copolymer. The characteristic peaks for succinimide appeared at 1781 and 1736 cm^{-1}, which were assigned to the asymmetric and symmetric carbonyl stretching, respectively. The weak absorption peak at 1812 cm^{-1} was assigned to the carbonyl stretching vibration in the NHS-activated ester [36] (Fig. 3a).

To introduce a disulfide bond into the pendant group of PEG-*b*-PNAS block copolymer, cystamine was reacted with NHS-activated ester to yield PEG-*b*-PAAm (Scheme 1). The formation of an insoluble product was observed as the reaction proceeded. After isolation, the product was insoluble in all organic solvents. The disulfide containing PEG-*b*-PAAm polymer was purified by dialysis in water. After freeze-drying, the purified polymer was collected as a fine powder, which was insoluble in common organic solvents. This insolubility was attributed to the intermolecular amidation of the free amine group in cystamine with a NAS moiety. FT-IR spectroscopy was used to identify the disulfide moieties in the cross-linked PEG-*b*-PAAm polymer. The loss of a succinimide group was confirmed by the disappearance of the three characteristic peaks at 1812, 1781, and 1736 cm^{-1} and

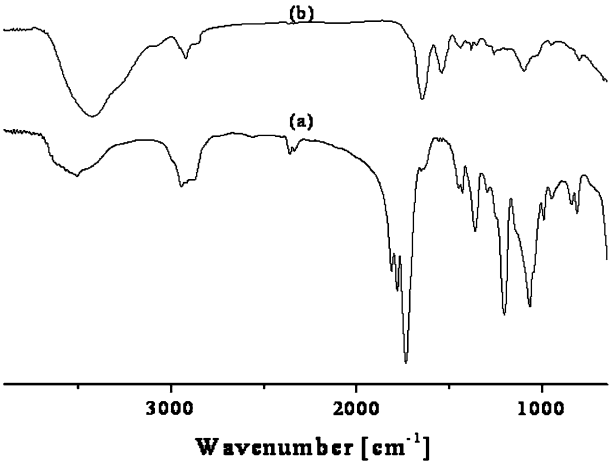


Fig. 3 FT-IR spectra of *a* PEG-*b*-PNAS diblock copolymer and *b* PEG-*b*-AAm diblock copolymer after introducing the disulfide groups

appearance of the two characteristic peaks for an amide group at 1646 and 1540 cm^{-1} for the C=O stretching and N–H bending vibrations, respectively (Fig. 3a, b). These results confirmed the successful introduction of disulfide moieties to the NHS-activated ester group in a PEG-*b*-PNAS block copolymer.

A thiol-containing block copolymer was easily prepared by the reductive cleavage of the disulfide bond in a PEG-*b*-PAAm cross-linked polymer. Many reagents for the chemical reduction of disulfides to thiol have been described, mostly for applications in the field of biochemistry [37, 38]. Dithiothreitol (2,3-dihydroxy-1,4-butanethiol, DTT) was used as the reducing agent to cleave the cross-linked disulfide bond in PEG-*b*-PAAm polymer. In this report, DTT was selected owing to its high efficiency and solubility in a range of solvents [22]. DTT was added to a dispersion of PEG-*b*-PAAm cross-linked polymer in deuterated DMF-d_7 at 50 °C under the nitrogen to avoid air oxidation of both polymeric thiol and DTT. The chemical transformation from disulfide to thiol was achieved after 17 h due to cleavage of the cross-linked polymers. This can be visualized easily by its physical appearance: there was an opaque solution containing PEG-*b*-PAAm particles. After adding the reducing agent, the solution became transparent and no detectable particles were observed, as confirmed by DLS measurement (data not shown). ^{1}H NMR spectroscopy was used to monitor the reduction of disulfide to thiol. Figure 4 shows the characteristic ^{1}H NMR peaks of PEG-*b*-PMEAAm diblock copolymer at 3.13 ppm (–NH–C**H**$_2$–CH$_2$–SH), 2.84 ppm (–NH–CH$_2$–C**H**$_2$–SH).

The thiol group of the PEG-*b*-PMEAAm block copolymer can be oxidized easily under mild conditions and then back to an opaque solution containing particles with disulfide bonds [39]. Reagents, such as iodine, $FeCl_3$, and oxygen from air, can be used [40]. Strong oxidizing agents should be avoided because they can oxidize the thiol group by air to the corresponding disulfide group and another oxidizing agent $FeCl_3$, which is known to convert thiol to disulfides efficiently. $FeCl_3$ was chosen as a mild oxidizing agent to avoid oxidation from the thiol group to sulfinate or sulfonate groups. After adding $FeCl_3$ to the solution containing the

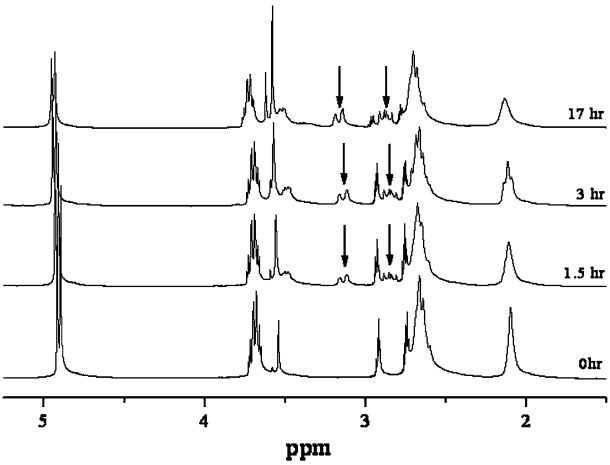


Fig. 4 ^{1}H NMR spectra during reduction from disulfide to thiol group

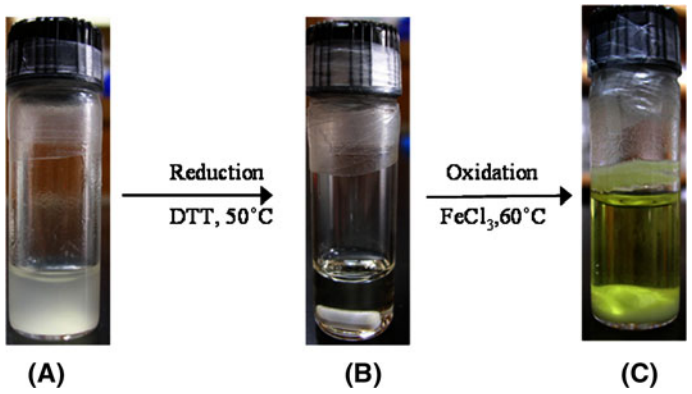


Fig. 5 Digital photographs of **a** dispersion of PEG-*b*-PAAm in DMF, **b** clear solution after reduction of disulfide groups, and **c** insoluble particle after oxidation

PEG-*b*-PNMEAAm block copolymer in DMF at 60 °C, precipitates formed at the bottom of the vial (Fig. 5c). This clearly indicates the formation of a disulfide bond.

Conclusions

A well-defined poly(ethylene glycol)-*b*-poly[(2-mercaptoethyl)acrylamide] diblock copolymer was synthesized by ATRP and subsequent post polymerization modification. Poly(ethylene glycol)-*b*-poly[*N*-(acryloxysuccinimide) was synthesized using PEG-Br and CuBr/2,2′-bipyridine as the macroinitiator and catalyst, respectively. Disulfide groups were introduced by a reaction with the NAS moieties of PEG-*b*-PNAS diblock copolymer and cystamine. The thiol-functionalized block copolymer was obtained by the reductive cleavage using DTT. The particle was formed reversibly by the oxidation of thiol using $FeCl_3$ through the formation of a disulfide bond. This can be visualized easily by its physical appearance: an opaque solution containing PEG-*b*-PAAm particles. After adding a reducing agent, the solution became transparent and no detectable particles were observed. This interesting feature of this polymer will allow tuning of these materials for specific applications, including protein conjugation or gene delivery.

Acknowledgments This study was supported by WCU (World Class University) program through the Korea Science and Engineering foundation funded by the Ministry of Education, Science and Technology (R33-2008-000-10035-0) and a grant from the Fundamental R&D Program for Core Technology of Materials funded by the Ministry of Knowledge Economy, Republic of Korea.

References

1. Jocelyn PC (1972) Biochemistry of the SH Group. Academic Press, London
2. Wang PH, Pan CY (2000) Safety of hormonal steroid contraceptive use for hepatitis B virus carrier women. Colloid Polym Sci 278:245–246. doi:10.1002/1099-1557(200005/06)9:3<245:AID-PDS500>3.0.CO;2-7

3. Thathagar MB, Beckers J, Rothenberg G (2003) Combinatorial design of copper-based mixed nanoclusters: new catalysts for Suzuki cross-coupling. Adv Synth Catal 345:979–985. doi:10.1002/adsc.200303045
4. Yu W, Liu M, Liu H, Ma X, Liu Z (1998) Preparation, characterization, and catalytic properties of polymer-stabilized ruthenium colloids. J Colloid Interface Sci 208:439. doi:10.1006/jcis.1998.5829
5. Gupta VK, Abbott NL (1997) Design of surfaces for patterned alignment of liquid crystals on planar and curved substrates. Science 276:1533–1536. doi:10.1126/science.276.5318.1533
6. Premachandran R, Banerjee S, John VT, McPherson GL, Akkara JA, Kaplan DL (1997) The enzymatic synthesis of thiol-containing polymers to prepare polymer–CdS nanocomposites. Chem Mater 9:1342–1347. doi:10.1021/cm960418p
7. Kim T, Crooks RM, Tsen M, Sun L (1995) Polymeric self-assembled monolayers. 2. Synthesis and characterization of self-assembled polydiacetylene mono- and multilayers. J Am Chem Soc 117:963–3967. doi:10.1021/ja00119a010
8. Lieb E, Hacker M, Tessmar J, Kunz-Schughart LA, Fiedler J, Dahmen C, Hersel U, Kessler H, Schulz MB, Goepferich A (2005) Mediating specific cell adhesion to low-adhesive diblock copolymers by instant modification with cyclic RGD peptides. Biomaterials 26:2333–2341. doi:10.1016/j.biomaterials.2004.07.010
9. Tessmar J, Mikos A, Gopferich A (2003) The use of poly(ethylene glycol)-block-poly(lactic acid) derived copolymers for the rapid creation of biomimetic surfaces. Biomaterials 24:4475–4486. doi:10.1016/S0142-9612(03)00345-4#
10. Andersson M, Elihn K, Fromell K, Caldwell KD (2004) Surface attachment of nanoparticles using oligonucleotides. Colloids Surf 34:165–171. doi:10.1016/j.colsurfb.2003.12.007
11. Calvo EJ, Danilowicz C, Lagier CM, Manrique J, Otero M (2004) Characterization of self-assembled redox polymer and antibody molecules on thiolated gold electrodes. Biosens Bioelectron 19:1219–1228. doi:10.1016/j.bios.2003.11.017
12. Kulkarni S, Schilli C, Mueller AHE, Hoffman AS, Stayton PS (2004) Reversible meso-scale smart polymer–protein particles of controlled sizes. Bioconjug Chem 15:747–753. doi:10.1021/bc034215k
13. Johnson PA, Levicky R (2003) Polymercaptosiloxane anchor films for robust immobilization of biomolecules to gold supports. Langmuir 19:10288–10294. doi:10.1021/la035102s
14. Kast CE, Bernkop-Schnurch A (2001) Thiolated polymers thiomers: development and in vitro evaluation of chitosan–thioglycolic acid conjugates. Biomaterials 22:2345–2352. doi:10.1016/S0142-9612(00)00421-X
15. Lele BS, Leroux JC (2002) Synthesis and micellar characterization of novel amphiphilic A–B–A triblock copolymers of *N*-(2-hydroxypropyl)methacrylamide or *N*-vinyl-2-pyrrolidone with poly(ε-caprolactone). Macromolecules 35:6714–6723. doi:0.1021/ma020433h
16. Kalarickal NC, Rimmer S, Sarker P, Leroux J-C (2007) Thiol-functionalized poly(ethylene glycol)-*b*-polyesters: synthesis and characterization. Macromolecules 40:1874–1880. doi:10.1021/ma062377g
17. Javakhishvili I, Hvilsted S (2009) Gold nanoparticles protected with thiol-derivatized amphiphilic poly(epsilon-caprolactone)-*b*-poly(acrylic acid). Biomacromolecules 10:74–81. doi:10.1021/bm800860t
18. Lowe AB, Sumerlin BS, Donovan MS, McCormick CL (2002) A highly active Suzuki catalyst for the synthesis of sterically hindered biaryls: novel ligand coordination. J Am Chem Soc 124:11562–11563. doi:10.1021/ja020556h
19. Mayadunne RTA, Rizzardo E, Chiefari J, Krstina J, Moad G, Postma A, Thang SH (2000) Living polymers by the use of trithiocarbonates as reversible addition–fragmentation chain transfer (RAFT) agents: ABA triblock copolymers by radical polymerization in two steps. Macromolecules 33:243–245. doi:10.1021/ma991451a
20. Schilli C, Lanzendoerfer MG, Mueller AHE (2002) Benzyl and cumyl dithiocarbamates as chain transfer agents in the RAFT polymerization of *N*-isopropylacrylamide. In Situ FT-NIR and MALDI–TOF MS investigation. Macromolecules 35:6819–6827. doi:10.1021/ma0121159
21. Sumerlin BS, Lowe AB, Stroud PA, Zhang P, Urban MW, McCormick CL (2003) Modification of gold surfaces with water-soluble (Co)polymers prepared via aqueous reversible addition–fragmentation chain transfer (RAFT) polymerization. Langmuir 19:5559–5562. doi:10.1021/la034459t
22. Nakayama M, Okano T (2005) Polymer terminal group effects on properties of thermoresponsive polymeric micelles with controlled outer-shell chain lengths. Biomacromolecules 6:2320–2327. doi:10.1021/bm050232w
23. Matyjaszewski K, Davis TP (eds) (2002) Handbook of radical polymerization. Wiley, Hoboken

24. Tsarevsky NV, Matyjaszewski K (2002) Reversible redox cleavage/coupling of polystyrene with disulfide or thiol groups prepared by atom transfer radical polymerization. Macromolecules 35:9009–9014. doi:10.1021/ma021061f
25. Tsarevsky NV, Matyjaszewski K (2005) Combining atom transfer radical polymerization and disulfide/thiol redox chemistry: a route to well-defined (bio)degradable polymeric materials. Macromolecules 38:3087–3092. doi:10.1021/ma050020r
26. Bontempo D, Heredia KL, Fish BA, Maynard HD (2004) Cysteine-reactive polymers synthesized by atom transfer radical polymerization for conjugation to proteins. J Am Chem Soc 126:15372–15373. doi:10.1021/ja045063m
27. Cossar BC, Fournier JO, Fields DL, Reynolds DD (1962) Preparation of thiols. J Org Chem 27:93–95. doi:10.1021/jo01048a024
28. Wardell JL (1974) Preparation of thiols. In: Patao S (ed) The chemistry of the thiol groups, part 1. Wiley, London, p 163
29. Yamashita K, Saba H, Tsuda K (1989) Synthesis and evaluation of thiol polymers. J Macromol Sci Chem A 26:1291–1304. doi:10.1080/00222338908052049
30. Yamashita K, Kimura Y, Saba H, Tsuda K (1991) Esterolysis of active esters by soluble thiol polymer. J Polym Sci Part A Polym Chem 29:777–779. doi:10.1002/pola.1991.080290521
31. Carrot G, Hilborn J, Hedrick JL, Trollss M (1999) Novel initiators for atom transfer radical and ring-opening polymerization: a new general method for the preparation of thiol-functional polymers. Macromolecules 32:5171–5173. doi:10.1021/ma9904565
32. Matyjaszewski K, Patten TE, Xia J (1997) Controlled/"living" radical polymerization. kinetics of the homogeneous atom transfer radical polymerization of styrene. J Am Chem Soc 119:674–680. doi:10.1021/ja963361g
33. Mammen M, Dahmann G, Whitesides GM (1995) Effective inhibitors of hemagglutination by influenza virus synthesized from polymers having active ester groups. Insight into mechanism of inhibition. J Med Chem 38:4179–4190. doi:10.1021/jm00021a007
34. Tian Y, Watanabe K, Kong X, Abe J, Iyoda T (2002) Synthesis, nanostructures, and functionality of amphiphilic liquid crystalline block copolymers with azobenzene moieties. Macromolecules 35:3739–3747. doi:10.1021/ma011859j
35. Hu Z, Liu Y, Hong C, Pan C (2005) Synthesis of well-defined glycoconjugate polyacrylamides via preactivated polymers prepared by ATRP. J Appl Polym Sci 98:189–194. doi:10.1002/app.22041
36. Frey BL, Corn RM (1996) Covalent attachment and derivatization of poly(L-lysine) monolayers on gold surfaces as characterized by polarization-modulation FT-IR spectroscopy. Anal Chem 68:3187–3193. doi:10.1021/ac9605861
37. Konigsberg W (1972) Reduction of disulfide bonds in proteins with dithiothreitol. Methods Enzymol 25:185–188. doi:10.1016/S0076-6879(72)25015-7
38. Singh R, Whitesides GM (1993) Thiol-disulfide exchange. In: Patai S, Rappoport Z (eds) The chemistry of sulfur-containing functional groups. Wiley, Chichester, pp 633–658
39. Marvel CS, Olson LE (1957) Polyalkylene disulfides. J Am Chem Soc 79:3089. doi:10.1021/ja01569a027
40. Capozzi G, Modena G (1974) Oxidation of thiols. In: Patai S (ed) The chemistry of the thiol group, part 2. Wiley, London, p 785