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Well-Defined Galactose-Containing Multi-Functional Copolymers and Glyconanoparticles for Biomolecular Recognition Processes

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ABSTRACT: We report here the syntheses of galactose-containing polymers *via* reversible addition−fragmentation chain transfer process. Diblock copolymers with one galactose-containing chain segment and one primary amine-containing or linear glucose-containing chain segment were prepared *via* chain extension technique. Primary amine pendant groups of the copolymer were further modified with biotinyl-*N*-hydroxysuccinimide ester. Subsequently, multifunctional glyconanoparticles were prepared and used in the study of biomolecular recognition processes. The biomolecular recognition of the biotin and galactose moiety on the surface of the glyconanoparticles toward avidin and *Ricinus communis* agglutinin lectin respectively was confirmed using UV−visible spectroscopy and diffractive optics techniques. It was found that both carbohydrate-carbohydrate and carbohydrate−protein interactions increased with increasing divalent salt (Ca^{2+} and Mn^{2+}) concentration.

Introduction

Carbohydrates, which represent an important class of biomolecules other than proteins and nucleic acids, have been the subject of intense research in the last decades.[1] With functions similar to those of natural carbohydrates, synthetic glycopolymers with specific pendant saccharide moieties can play a significant role in pathological and biological events[2] *via* multivalent carbohydrate−protein interactions.[3] More recently, some carbohydrate−protein/cell interaction studies[4] and applications of glycopolymers, such as in antivirus/bacteria[5] and gene delivery,[6] were reported. Carbohydrate-based polymers with different properties were also synthesized, including biodegradable,[7] thermosensitive, and acid-degradable core-cross-linked glyconanoparticles,[8] with neuroactivity[9] and with chiroptical properties.[10]

Based on the increasing understanding of the roles of carbohydrates in biological systems, synthetic carbohydrate-based materials, particularly, glycopolymers and glyconanoparticles are receiving enormous attention. Various methods have been employed in their preparation. As presented in the reviews,[11] with the development of living polymerization technique, such as living ionic polymerization,[12] ring-opening metathesis polymerization,[13] nitroxide-mediated polymerization[14] and atom transfer radical polymerization (ATRP),[15] well-defined glycopolymers can be synthesized. However, most of these synthetic processes either involved protecting group chemistry or suffered from a limited range of monomers. A direct strategy for the preparation of carbohydrate-based vinyl monomers and polymers *via* ATRP without using any protecting group chemistry was first reported by Narain et al.[16] In 2008, an *N*-acetyl-D-glucosamine containing biohybrid material was successfully synthesized using ATRP by Maynard's research group.[17] More recently, Dong et al. reported the preparation of biodegradable star-shaped poly(ε-caprolactone-*b*-lactobionamidoethyl methacrylate) biohybrids *via* ATRP.[18] However, high hydrophilic nature of carbohydrate-based monomers/polymers restricts the application of ATRP due to the serious deactivation of ATRP catalyst in high ratio of water to organic solvent. To overcome this disadvantage, protecting group chemistry was often taken back into consideration, which can increase the solubility of carbohydrates in pure organic solvent. In this regard, the newest living polymerization technique, reversible addition−fragmentation chain transfer (RAFT) process,[19] seems to have more potential. Although the RAFT chain transfer agent (CTA) will also undergo slow hydrolysis, either during homopolymerization or copolymerization, the resulting polymer chains can still grow in a control manner using a high ratio of water to organic solvent. Direct polymerization of 2-methacryloxyethyl glucoside *via* RAFT in aqueous phase employing 4-cyanopentanoic acid dithiobenzoate (CTP) as CTA was first reported by McCormick research group.[20] More recently, a RAFT polymerization of 2-lactobionamidoethyl methacrylate (LAMA) was reported by our group.[21]

Different types of carbohydrates such as lactose,[22,23] glucoside,[24] mannose,[25] and galactose[26] have been used as precursors to synthesize glycopolymers. However there are few reports on the synthesis and biomolecule recognition of well-defined synthetic glycopolymers with different bioactive pendant groups on the same polymer chain. To the best of our knowledge, the first example was reported by Roy et al. in 1992 and involved lactose and biotin segments,[27] as well as Thomsen−Friedenrich carbohydrate antigen (T-Ag), Galβ(1−3)-GalNAcα and biotin segments.[28] However, these segments were copolymerized randomly. Another example was reported by Tsuchida et al. using sialyllactose derivatives as monomers.[29] Random copolymers with Galα1→3Gal and α-mannosyl were reported by Wang et al. in 1999[30] and more recently, mannoside and galactoside moieties were introduced randomly to polymer *via* "click" chemistry.[31] Only one example of well-defined diblock glycopolymer with two different types of carbohydrate moieties was reported by Albertin et al.[32] However, the study of the interaction between biomolecules and those well-defined copolymers have not been reported.

The affinity of glycopolymer to lectin increased with the number-average degree of polymerization (DP_n), and therefore the number of sugar moieties.[33] Such phenomenon is called

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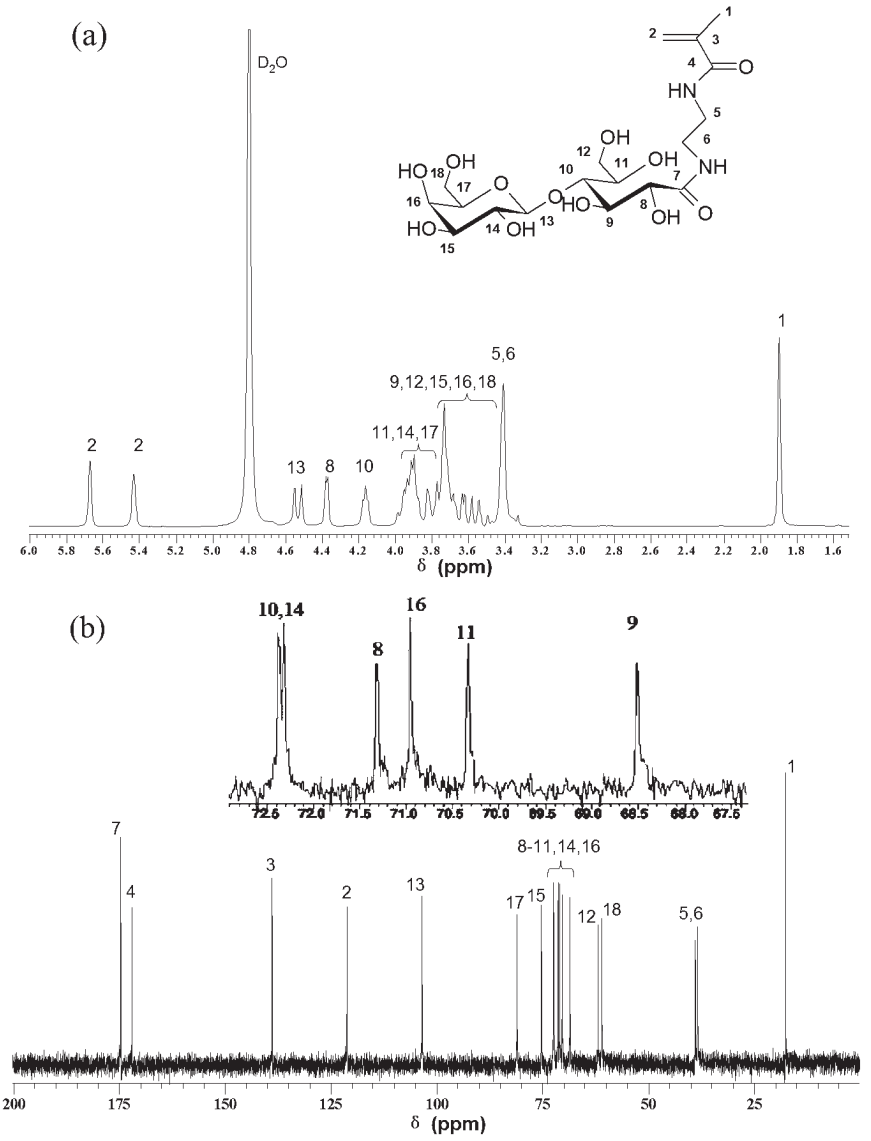


Figure 1. Assigned ^{1}H (a) and ^{13}C (b) NMR (D_2O) spectra for the 2-lactobionamidoethyl methacrylamide monomer.

glycocluster effects,[34] which was contributed by the multiple binding to lectin[35] and cooperativity of the glycopolymer.[36] Therefore, the control of the DP_n of glycopolymers seems to be very important. Accordingly, compared with end-group, pendant group functionalization may have higher efficiency in bioconjugation applications. Having access to RAFT technique, well-defined glycopolymers as well as diblock copolymers can be tailor-made. This paper reports a novel galactose-containing glycomonomer with amide linkage. Well-defined galactose-containing homopolymer as well as copolymers with different types of pendant groups for bioconjugation application were synthesized *via* RAFT macro-CTA technique. The interactions between lectins and surface-functionalized gold nanoparticles with these copolymers were investigated.

Experimental Section

Materials. Lactobionolactone,[16] 2-aminoethyl methacrylamide hydrochloride (AEMA),[37] 3-aminopropyl methacrylamide hydrochloride (APMA),[37] 2-gluconamidoethyl methacrylamide (GAEMA)[38] and biotinyl-*N*-hydroxysuccinimide ester (Biotin-NHS)[39] were synthesized according to previous work. 4,4′-Azobis(4-cyanovaleric acid) (ACVA, 97%) was purchased from Acros Organics and used as received. The chain transfer agent, 4-cyanopentanoic acid dithiobenzoate,[40] and *S*,*S*′-bis(α,α′-dimethyl-α′′-acetic acid)trithiocarbonate (CTAd)[41] were synthesized as previously described. HPLC grade methanol and reagent grade *N*,*N*′-dimethylformamide (DMF) were purchased from Caledon Chemicals. All proteins used in this study were purchased from Sigma-Aldrich. Doubly distilled deionized water was used in all experiments.

Synthesis of 2-Lactobionamidoethyl Methacrylamide (LAEMA). LAEMA was prepared according to the method reported by Narain et al.[16] with slight modification. Fresh prepared lactobionolactone (10.0 g, 29.4 mmol) was immediately dissolved in 20.0 mL of methanol at room temperature before the addition of 15.0 mL of methanol solution of AEMA (5.8 g, 35.3 mmol) and 10.0 mL of triethylamine. The pH value of this solution is about 8. The mixture was kept stirring at room temperature until white precipitate formed. Final product was precipitated in 2-propanol followed by washing with acetone, dried under vacuum. Final yield of LAEMA was ~60%. Characterization of the LAEMA monomer by both ^{1}H and ^{13}C NMR spectroscopy (Figure 1) and also mass spectroscopy (see Supporting Information Figure S1) confirmed its high purity.

RAFT Polymerization of 2-Lactobionamidoethyl Methacrylamide. RAFT polymerizations of LAEMA were achieved at 70 °C, employing 4,4′-azobis(4-cyanovaleric acid) as the initiator and

4-cyanopentanoic acid dithiobenzoate as the chain transfer agent. A typical protocol is as follows. In a 10-mL Schlenk tube, LAEMA (1.0 g, 2.13 mmol) was dissolved in double distilled water (7.0 mL) followed by the addition of 1.0 mL CTP (0.008 g, 0.027 mmol) and ACVA (0.004 g, 0.013 mmol) in DMF solution. After degassing via four freeze−thaw cycles, the flask was placed in an oil bath for polymerization at 70 °C for 5 h. The conversion was determined by ^{1}H NMR spectroscopy using D_2O as solvent and by comparing the vinyl resonance (appearing between 5.3 and 5.8 ppm) to the methyl resonance of the polymer (appearing between 0.5 and 1.4 ppm) (Figure S2). Number average molecular weight (M_n) and molecular weight distributions (M_w/M_n) were obtained from aqueous gel permeation chromatography (GPC) after the polymer was precipitated in acetone (conversion ~ 87%; M_n = 26 500 g/mol; M_w/M_n = 1.31; Figure 3; Table 2).

Self-Blocking Experiment of Poly(LAEMA). LAEMA was first polymerized with CTP and quenched after 4 h by cooling in an ice bath, followed by freezing in liquid nitrogen and being exposed to air. The resulting polymer was precipitated in acetone and the residual monomer was removed by washing with methanol. Subsequently, fresh prepared poly(LAEMA) (M_n = 8600 g/mol; M_w/M_n = 1.12) was used as macro-CTA to conduct the self-blocking experiment immediately. Additional LAEMA monomer (0.10 g, 0.21 mmol) and poly(LAEMA) macro-CTA (0.10 g, 0.012 mmol) were first dissolved in 1.8 mL doubly distilled water followed by the addition of 0.2 mL ACVA (0.001 g, 0.004 mmol) DMF stock solution. After degassing *via* four freeze−thaw cycles, the flask was placed in a preheated oil bath for 14 h at 70 °C. The resulting polymer was precipitated in acetone (yield ~ 90%; M_n = 15 000 g/mol; M_w/M_n = 1.24; Table 4, entry 1).

RAFT Diblock Copolymerization of 2-Lactobionamidoethyl Methacrylamide with Heterogeneous Monomers. LAEMA was first polymerized using CTP as chain transfer agent and ACVA as initiator and quenched at a proper time. A heterogeneous monomer, AEMA (0.10 g, 0.61 mmol) or GAEMA (0.10 g, 0.33 mmol), was added to poly(LAEMA) solution (0.10 g in 1.8 mL double distilled water) followed by the addition of 0.2 mL ACVA (0.001 g, 0.004 mmol) DMF stock solution. After being degassed via four freeze−thaw cycles, the flask was placed in a 70 °C oil bath. The resulting diblock copolymer poly(LAEMA-*b*-AEMA) or poly(LAEMA-*b*-GAEMA) was precipitated in acetone. The GPC results of these diblock copolymers were shown in Figure 6 and Table 3 and ^{1}H NMR spectra were shown in Supporting Information Figure S4.

RAFT Diblock Copolymerization of 3-Aminopropyl Methacrylamide Hydrochloride with 2-Lactobionamidoethyl Methacrylamide. APMA was first polymerized with CTP according to the previous report.[37] Subsequently, poly(APMA) (M_n = 5500 g/mol; M_w/M_n = 1.09) was used as macro-CTA to synthesize poly(APMA-*b*-LAEMA) diblock copolymer. As the second monomer, LAEMA (1.60 g, 3.426 mmol) was added to poly(APMA) solution (0.480 g, 0.087 mmol in 8.0 mL double distilled water) followed by the addition of 0.5 mL ACVA (0.009 g, 0.032 mmol) DMF stock solution. After degassing *via* four freeze−thaw cycles, the flask was placed in a preheated oil bath for 15 h at 70 °C. The diblock copolymer poly(APMA-*b*-LAEMA) was precipitated in acetone followed by washing with methanol (yield ~ 90%; M_n = 20 500 g/mol; M_w/M_n = 1.21; Figure 6, Table 3; ^{1}H NMR spectrum was shown in Supporting Information Figure S4)

Modification of Poly(APMA-*b*-LAEMA) with Biotinyl-*N*-hydroxysuccinimide Ester. Biotinyl-*N*-hydroxysuccinimide ester was synthesized according to previous work[39] by reacting D-biotin with *N*-hydroxysuccinimide. Subsequently, biotinyl-*N*-hydroxysuccinimide ester (0.052 g, 0.15 mmol) was dissolved in 4.0 mL H_2O/DMF (3:1, v/v) mixed solvent followed by the addition of 4.0 mL aqueous solution (pH = 9) containing 0.100 g poly($APMA_{31}$-*b*-$LAEMA_{32}$). The mixture was stirred overnight at room temperature. The insoluble substance was filtered out and the solvent was removed by rotatory evaporation. Residual biotinyl-*N*-hydroxysuccinimide ester was washed with $CHCl_3$/DMSO (20:1, v/v) mixture. Successful modification was confirmed by ^{1}H NMR (Figure 8) (conversion: ~52%).

Synthesis of Functionalized Gold Nanoparticles. Gold nanoparticles were synthesized according to the method described by Brust et al.[42] As stabilizer, different types of RAFT prepared glyco-copolymers (0.001 g, 0.049 μmol of poly($APMA_{31}$-*b*-$LAEMA_{32}$), 0.008 g, 0.482 μmol of poly(LAEMA-*b*-GAEMA) and 0.001 g, 0.042 μmol of poly(BAPMA-*b*-LAEMA)) were first dissolved in 2.0 mL deionized water. 1.0 mL $HAuCl_4$ aqueous solution (2.000 g/L) was added to the polymer solutions. The mixture was stirred at room temperature for 20 min followed by the dropwise addition of $NaBH_4$ aqueous solution (4.250 g/L) until the color of the solution changed into dark red. The mixtures were kept stirring at room temperature overnight.

4-Hydroxyazobenzene 2-Carboxylic Acid (HABA)/Avidin Binding Assay. HABA/avidin binding assay was conducted according to the method reported by Green et al.[43] Formula for calculation of the biotin concentration:

$$\Delta A_{500} = 0.9 \times A_{\text{HABA/avidin}} + A_{\text{AuNPs}} - A_{\text{HABA/avidin + AuNPs}}$$

$$\mu\text{mol of biotin/mL} = 10 \times (\Delta A_{500}/34)$$

Lectin Recognition. The lectin recognition activity was analyzed by changes in the turbidity of AuNPs solution with time at room temperature after the addition of various types of lectins. The *Ricinus communis* agglutinin from castor bean (RCA_{120}) and Wild type Streptavidin recognitions were conducted in pH 7.4 phosphate buffer solution (PBS). Concanavalin A (Con A) and Lectin from Artocarpus Integrifolia (Jacalin) recognitions were conducted in pH 7.0 PBS containing 1.0×10^{-4} mmol/mL Ca^{2+}, 1.0×10^{-4} mmol/mL Mn^{2+} and 0.1 mmol/mL NaCl unless other notice. Bovine serum albumin (BSA) was used as control experiment for nonspecific protein adsorption. UV−visible spectra (300−800 nm) were recorded at room temperature.

Characterization. ^{1}H and ^{13}C NMR spectra of the monomers and polymers were recorded on a Varian 200 MHz instrument.

The monomers were analyzed by an Agilent HPLC 1100 interfaced with an Electro-Spray Ionization Agilent Mass Spectrometer Model 6120 with a Chemstation data system LC-MSD B.03.01.

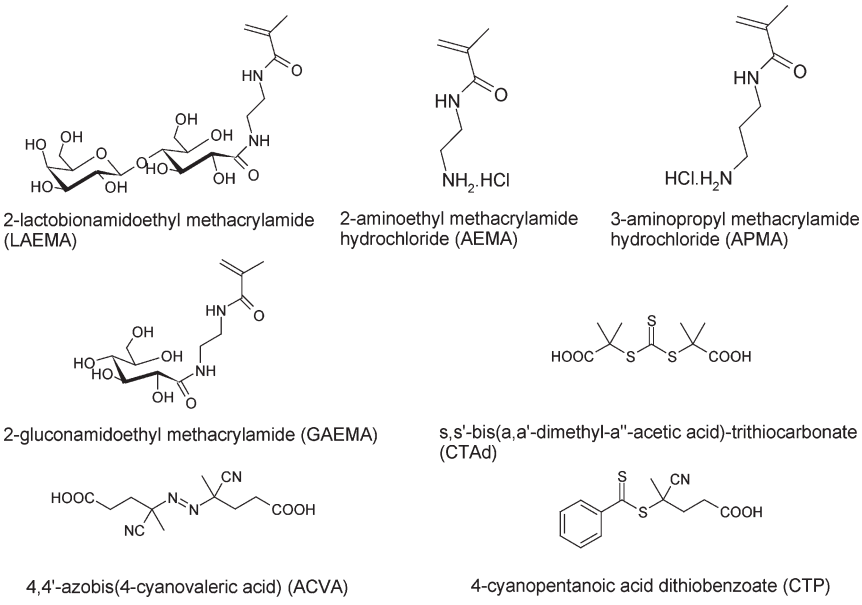
M_n and M_w/M_n were determined at room temperature by Viscotek conventional GPC system equipped with Viscotek model 250 dual detector (refractometer/viscometer), using aqueous eluents at a flow rate of 1.0 mL/min. Two Waters Ultrahydrogel linear WAT011545 columns (pore size: blend, exclusion limit = 7×10^6) were used. The following two GPC protocols were used in this work.

Aqueous GPC at Neutral pH. This protocol was used for LAEMA homopolymers and LAEMA-GAEMA copolymer. Chromatograms were recorded from the conventional Viscotek GPC system using a 0.25 M sodium nitrate as eluent. Seven near-monodisperse polymaltotriose (Pullulan) standards (M_w = 5900−404000 g mol^{-1}) were used for calibration.

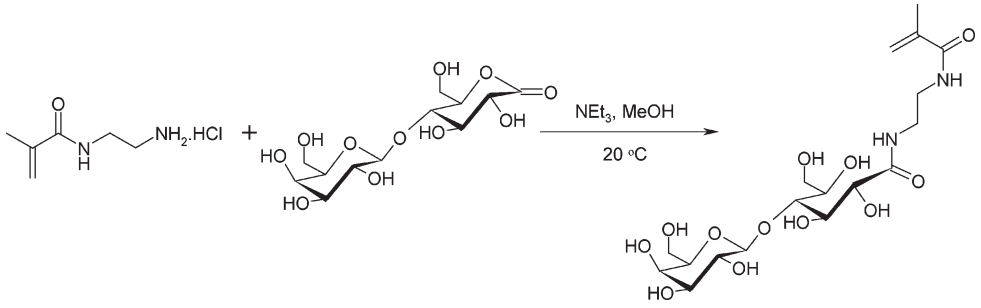
Aqueous GPC at Low pH. Chromatograms for the LAEMA−APMA and LAEMA−AEMA diblock copolymers were obtained from the conventional Viscotek GPC system using a 0.50 M sodium acetate/0.50 M acetic acid buffer as eluent. Seven near-monodisperse polymaltotriose (Pullulan) standards (M_w = 5900−404000 g mol^{-1}) were used for calibration. Six near-monodisperse polyethylene oxide (PEO) standards (M_w = 1020−96750 g mol^{-1}) were used for the calibration of APMA homopolymers.

Dynamic light scattering measurements were performed at room temperature using a Viscotek dynamic light scattering instrument (DLS) having a He−Ne laser at a wavelength of 632 nm and pelltier temperature controller.

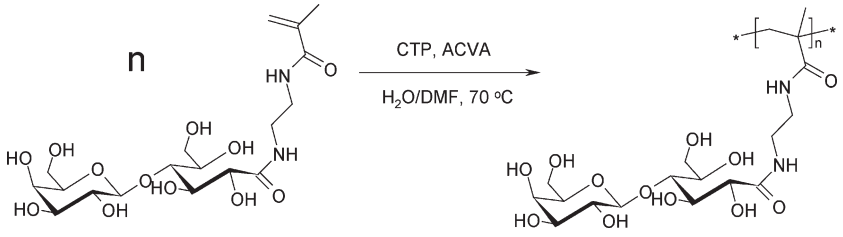
Scheme 1. Chemical Structures of Monomers, Chain Transfer Agents, and Initiator Used in This Study



Scheme 2. Synthesis of 2-Lactobionamidoethyl Methacrylamide



Scheme 3. Reversible Addition−Fragmentation Chain Transfer Polymerization of 2-Lactobionamidoethyl Methacrylamide



Carbohydrate−lectin interactions were investigated by BioChrom Ultrospec 2100 pro UV−visible spectrophotometer and Axela Biosensors Inc. dotLab system (Model: AL-1) with avidin sensor kit.

Results and Discussion

Synthesis of LAEMA Glycomonomer. As a kind of oligosaccharide aldonic acid, lactobionic acid comprises a galactose and a gluconic acid unit which linked each other *via* an enzymatically hydrolyzable ether bond. Moreover, it has been shown that galactose moiety has a high and specific affinity to liver cells. Studies of affinity of galactose-containing material with lectins,[44,45] *in vitro*[46] and *in vivo*[47] liver cells have been reported. Therefore, lactobionic acid was selected as a carbohydrate precursor to prepare polymerizable glycomonomer. LAEMA was readily synthesized in reasonable yields in anhydrous methanol at room temperature by the reaction of AEMA with lactobionolactone (Scheme 2). This ring-opening of cyclic lactone with primary amine eliminates the involvement of protecting group chemistry. Compared with the synthesis of LAMA,[16] LAEMA was obtained in a slightly lower yield and its synthesis process required longer reaction time, at least 12 h even at ~35% w/v reaction concentration. These may be due to the lower activity of AEMA primary amine group than that of 2-aminoethyl methacrylate. Higher reaction temperature (e.g., 50 °C) can reduce the reaction time. However, in that case, inhibitor is required to prevent the self-initiation of the polymerization. Interestingly, in contrast to monosaccharide GAEMA, disaccharide nature of LAEMA does not reduce its solubility in DMF. In other words, LAEMA is more soluble than GAEMA not only in water, but also in DMF.

Tsuchida et al. reported a comparison of two glycopolymers with the same sugar moiety but different linkage modes and different backbones.[48] It was shown that the *O*-β-glycoside-bearing polymer has higher affinity to lectin than the *N*-β-glycoside-bearing polymer. However, it is still unclear whether the difference of backbones affects the bioaffinity. Due to the slightly structural difference, amide linkage against ester linkage (Supporting Information Scheme S1), it may be of interest to make a comparison between LAEMA and LAMA as well as their homopolymers, which may offer an opportunity to study the effect of polymer backbone on sugar bioaffinity.

Homopolymerization. The polymerizations of LAEMA were conducted *via* the RAFT technique (Scheme 3). 4-Cyanopentanoic acid dithiobenzoate is the most commonly used and well investigated chain transfer agent with reasonable water solubility. On the other hand, it is compatible with a wide range of monomers that makes it easier to synthesize multifunctional copolymers *via* macro-CTA chain extension technique, although it could undergo hydrolysis and aminolysis.[49] Successful polymerizations of 2-(β-D-galactosyloxy)ethyl methacrylate, methyl[24,50] 6-*O*-methacryloyl-α-D-glucoside,[32] 6-*O*-methacryloyl mannose,[51] methyl 6-*O*-methacryloyl-α-D-mannoside[32] and 2-methacryloxyethyl glucoside,[32] using CTP as chain transfer agent were reported by Cameron, Albertin, Barner-Kowollik, Stenzel, and Davis et al. Application of CTP to the polymerization of acrylamide derivatives with α-mannose and *N*-acetyl-β-glucosamine was also reported by the Miura research group.[52] In order to keep the nature of CTP leaving group, ACVA was selected as free radical source which can generate initiator radicals I$^\bullet$ with the same chemical structure as CTP leaving group radical R$^\bullet$ (I$^\bullet$ = R$^\bullet$ = $(CH_3)C^\bullet(CN)(CH_2)_2-$COOH) (Scheme 1). The difference of soluble nature of CTP, ACVA, LAEMA monomer and corresponding polymer rendered the polymerization impossible to be conducted in ether pure water or pure organic solvent. Therefore, H_2O/DMF was selected as cosolvent system to ensure the well dissolution of all chemicals, especially the resulting polymer.

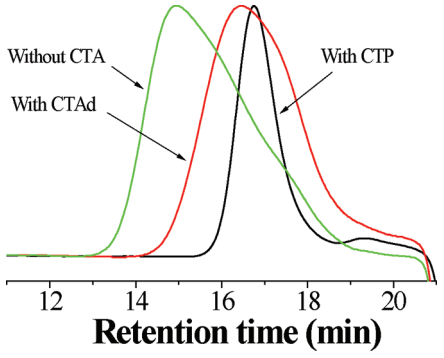


Figure 2. Aqueous gel permeation chromatography traces for the homopolymerization of 2-lactobionamidoethyl methacrylamide without ([LAEMA]:[CTA]:[ACVA] = 82:0:1; concentration of LAEMA = 12.5% w/v; solvent, H_2O/DMF = 7:1) and with different chain transfer agents ([LAEMA]:[CTP or CTAd]:[ACVA] = 82:2:1; concentration of LAEMA = 12.5% w/v; solvent, H_2O/DMF = 7:1).

The polymerization temperature was set to 70 °C mainly due to the half-life of the ACVA initiator (10 h at 69 °C in water). Higher temperature will accelerate the hydrolysis of CTA, while lower temperature will slow down the polymerization. In our case, acidic to neutral pH values do not have obvious effect on the resulting polymers. However, in the presence of sodium acetate salt, the solution color was found to change into yellow more rapidly. Hence, no buffer solution was employed. Under this condition, the first series of polymerizations were conducted with and without the presence of water-soluble chain transfer agents. The GPC results clearly show that CTP has the highest efficiency (Figure 2). Without chain transfer agent, a much broader molecular weight distribution was observed and molecular weight was found about twice higher (~20 kDa against ~10 kDa). In the presence of chain transfer agents, the molecular weight were found close (~10 kDa with CTAd against ~13 kDa with CTP), however, the polydispersity was much higher when CTAd was employed (Table 1). Detailed kinetic studies were carried out using CTP as chain transfer agent and ACVA as initiator. A clear shift of GPC traces to higher molecular weight was observed. Pseudo first order kinetics and linear evolution of M_n with conversion indicated the control of the polymerization (Figure 3, Table 2). Surprisingly, the polymerization of LAEMA was found fast, about twice as fast as GAEMA (Figure 4), in a similar reaction condition ([monomer] = 0.408 M; [CTP] = 0.008 M; [ACVA] = 0.004 M; solvent, 7:1, H_2O:DMF). Different degrees of polymerization were also targeted in the experiment. GPC results indicated that polymers with different molecular weights were successfully obtained (Figure 5). A long inhibition period was still observed in the polymerization and it seems to be prolonged as CTP concentration decreases (see Supporting Information). As mentioned above, because of the same chemical structure of ACVA radical (I$^\bullet$) and CTP leaving group radical (R$^\bullet$), there is no chance to change the chemical structure of the chain transfer agent reactive group at the polymer chain end during the course of the polymerization.

Copolymerization. Different types of synthetic galactose-containing copolymers were mainly synthesized *via* RAFT chain extension from macro-CTA. Primary amine based monomers, such as AEMA or APMA, were chosen to conduct the chain extension experiments due to the complexation ability with DNA[38] and the possibility of post-modification. As a different type of carbohydrate, GAEMA was selected as second monomer for the investigation in bioconjugation. Clear shift of monomodal molecular weight distribution to higher molecular weight indicated successful chain extension experiments (Figure 6, Table 3). ^{1}H NMR also confirmed the successful blocking experiment with different monomers (Supporting Information Figure S4).

A tail to low molecular weight was observed in all cases. Similar observation was previously reported.[32] Therefore, some further experiments were conducted to examine the efficiency of macro-CTA. Due to the reason that dialysis of macro-CTA[24] might fractionate the sample (removing the "dead chains"), which could minimize the tail of resulting copolymers, precipitation method was used to purify the

Table 1. Summary of Synthetic Parameters for Homopolymerization of 2-Lactobionamidoethyl Methacrylamide without ([LAEMA]:[CTA]:[ACVA] = 82:0:1; Concentration of LAEMA = 12.5% w/v; solvent, H_2O/DMF = 7:1) and with Different Chain Transfer Agents ([LAEMA]:[CTP or CTAd]:[ACVA] = 82:2:1; Concentration of LAEMA = 12.5% w/v; solvent, H_2O/DMF = 7:1)

entry	CTA	polymerization time (h)	convn (%) ^{1}H NMR	$M_{n,GPC}$ (g/mol)	M_w/M_n
1	N/A	15	95	21700	4.52
2	CTAd	15	86	10300	2.53
3	CTP	23	89	13700	1.16

macro-CTA in this study. On the other hand, self-blocking experiments were employed in the experiment to exclude any other side effects coming from the compatibility of the second chain segment while conducted the polymerization and analysis. In the first self-blocking experiment, LAEMA was first polymerized with CTP and the ratio of chain transfer agent to initiator was set to 3 and the polymerization was quenched after 4 h at ~50% conversion. According to previous kinetic study,[38] "living" status can be maintained up to 8 h using CTP as chain transfer agent. Afterward, obvious color change can be observed indicating the potential hydrolysis of CTP. On the other hand, in RAFT polymerization, deceleration in semilogarithmic plot versus time was usually found after 80% conversion, which may be due to the radical terminations. Therefore the polymerization was quenched at interim to minimize side reactions. However, a tail of the resulting self-blocking polymer to low molecular weight was still observed. Although polydispersity of the resulting self-blocking polymer was still low (less than 1.30), an increase in polydispersity was still found compared to macro-CTA (1.24 against 1.12, see Table 4, entry 1). Therefore, two more self-blocking experiments were conducted. First, macro-CTA was synthesized employing a low ratio of CTA to initiator (1 to 2) and then such a macro-CTA was used to conduct the self-blocking experiment. The mixture containing macro-CTA, LAEMA monomer and ACVA was kept stirring in a 70 °C oil bath for 18 h. The GPC result of self-blocking polymer showed that there is no obvious difference of polydispersity ($M_w/M_n = 1.25$ against 1.24). It should be noted that low polydispersity ($M_w/M_n = 1.10$) of macro-CTA was still obtained in the condition of low ratio of CTA to initiator (see Table 4, entry 2), and second, the polymerization was accelerated by the addition of a large amount of initiator. High conversion (> 90%) was obtained after 3 h of polymerization. However, it seems that there is no obvious effect on the efficiency of macro-CTA in the chain extension experiment. In the second experiment, macro-CTA was synthesized using the same ratio of CTA to initiator (3:1) as Table 4, entry 1, but the polymerization was kept continuous for 15 h. Such macro-CTA was subsequently used to conduct the self-blocking experiment. The GPC trace of resulting self-blocking polymer shows only a slight shift to higher molecular weight. Moreover, an obvious higher molecular weight shoulder was observed (Figure 7) and polydispersity increased obviously (1.39 in Table 4, entry 3, against 1.24 in entry 1). In aqueous RAFT polymerization, longer polymerization time mainly results in high conversion and deactivation of CTA. Radical

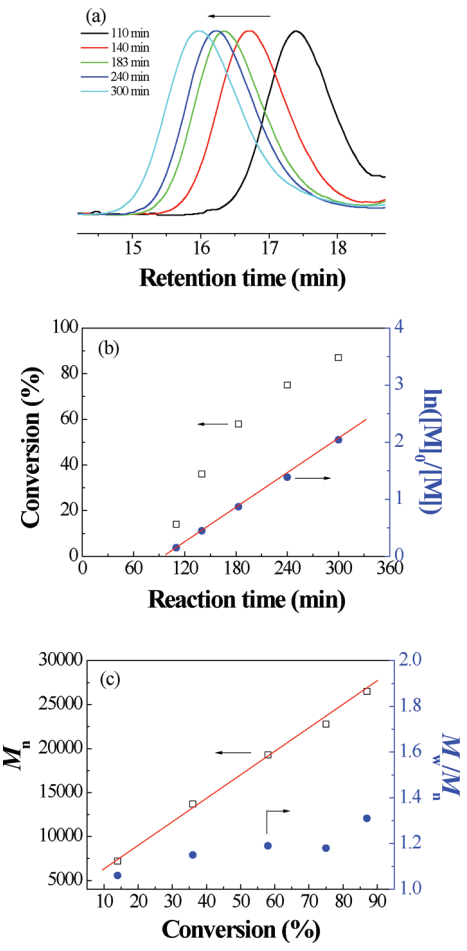


Figure 3. Kinetic study for the reversible addition−fragmentation chain transfer polymerization of 2-lactobionamidoethyl methacrylamide. (a) aqueous gel permeation chromatography trace indicating the evolution of M_n with time; (b) semilogarithmic and conversion plot versus time; (c) evolution of M_n and M_w/M_n with conversion. ([LAEMA]:[CTP]:[ACVA] = 164:2:1; concentration of LAEMA = 12.5% w/v; solvent, H_2O/DMF = 7:1).

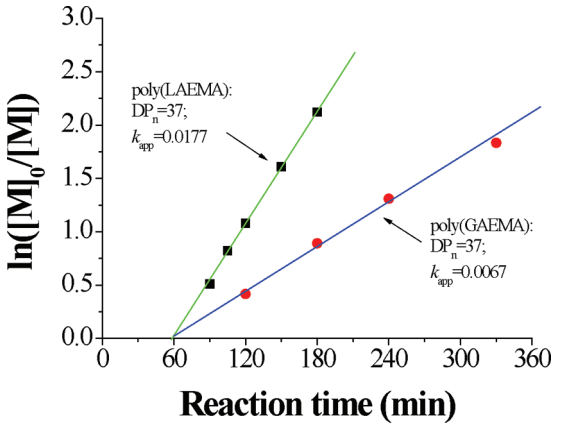


Figure 4. Semilogarithmic plot versus time for kinetic study of reversible addition−fragmentation chain transfer polymerizations of 2-lactobionamidoethyl methacrylamide and 2-gluconamidoethyl methacrylamide. ([Monomer]:[CTP]: [ACVA] = 100:2:1, [Monomer] = 0.4 M, solvent: H_2O/DMF = 7:1).

Table 2. Summary of Synthetic Parameters for the Homopolymerization of 2-Lactobionamidoethyl Methacrylamide at 70 °C Using 4-Cyanopentanoic Acid Dithiobenzoate as Chain Transfer Agent and 4,4′-Azobis(4-cyanovaleric acid) as Initiator ([LAEMA]:[CTP]:[ACVA] = 164:2:1; Concentration of LAEMA = 12.5% w/v; solvent, H_2O/DMF = 7:1)

entry	polymerization time (min)	convn (%) ^{1}H NMR	$M_{n,th}$[a]	$M_{n,GPC}$ (g/mol)	M_w/M_n
1	110	14	4200	7200	1.06
2	140	36	10300	13700	1.15
3	180	58	16500	19300	1.19
4	240	75	21200	22800	1.18
5	300	87	24600	26500	1.31

[a] $M_{n,th} = [LAEMA]_0/([CTP]_0 + [ACVA]_0) \times MW_{LAEMA} \times$ conversion $+ MW_{CTP}$.

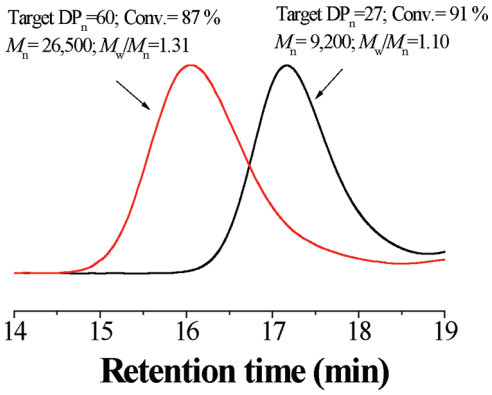


Figure 5. Aqueous gel permeation chromatography traces for the reversible addition−fragmentation chain transfer polymerization of 2-lactobionamidoethyl methacrylamide with different target degrees of polymerization. ([CTP]:[ACVA] = 2:1; concentration of LAEMA = 12.5% w/v; solvent, H_2O/DMF = 7:1).

termination reactions become the main reaction in the system after the polymerization goes to high conversion, which may cause significant "dead chains" and affect the subsequent chain extension. However, the result of Table 4, entry 2, experiment shows that such "radical termination" may not be the main reason. Hence, deactivation of CTA is proposed as the main reason for the broadening of the GPC curve in blocked copolymerization. To conclude the results of these three experiments, in aqueous RAFT polymerization, reaction time for the synthesis of macro-CTA should be taken into consideration.

Modification of Poly(APMA-*b*-LAEMA) with Biotinyl-*N*-hydroxysuccinimide Ester. Compared with end-group, pendant group functionalization may offer an opportunity to synthesize biomaterials with higher affinity. The possibility to prepare diblock glycopolymers carrying different bioactive functional groups on the two blocks may add a level of the design of the biomaterials used in biomedical studies. As an example, biotin moieties were introduced to the well-defined diblock glycopolymer by the coupling reaction of primary amine pendant groups on poly($APMA_{31}$-*b*-$LAEMA_{32}$) with biotinyl-*N*-hydroxysuccinimide ester (Scheme 4). Commercially available D-biotin was first converted to biotinyl-*N*-hydroxysuccinimide ester which has high reactivity to primary amine. Due to the difference in solubility of biotinyl-*N*-hydroxysuccinimide ester and poly($APMA_{31}$-*b*-$LAEMA_{32}$), mixed solvent (H_2O/DMF) was used. The pH value of the solution was adjusted to 9 to ensure that the primary amine groups were deprotonated. The washing solvent, chloroform/dimethylsulfoxide (20:1,v/v), used in the purification was carefully chosen to remove unreacted residual biotinyl-*N*-hydroxysuccinimide ester. By comparing the integrations of biotin proton *a* (appearing between 2.1 and 2.5 ppm), biotin protons *f, g* (appearing between 4.3 and 4.7 ppm) and carbohydrate proton *12* (appearing between 4.1 and 4.3 ppm), and carbohydrate protons *10, 15* (appearing between 4.3 and 4.7 ppm), the ^{1}H NMR spectra (Figure 8) indicated that about ~50% primary amine groups were converted to biotin moieties. However, 4-hydroxyazobenzene 2-carboxylic acid (HABA)/avidin binding assay[43] showed only about 30% of the primay amine pendant groups were modified (Figure 9). It should also be noted that the modification was carried out on polymers, which makes it difficult to accurately assess the biotin content by NMR spectroscopy or HABA/Avidin assay. HABA/avidin binding assay can only detect available biotin (unhindered) for conjugation. Therefore, this method

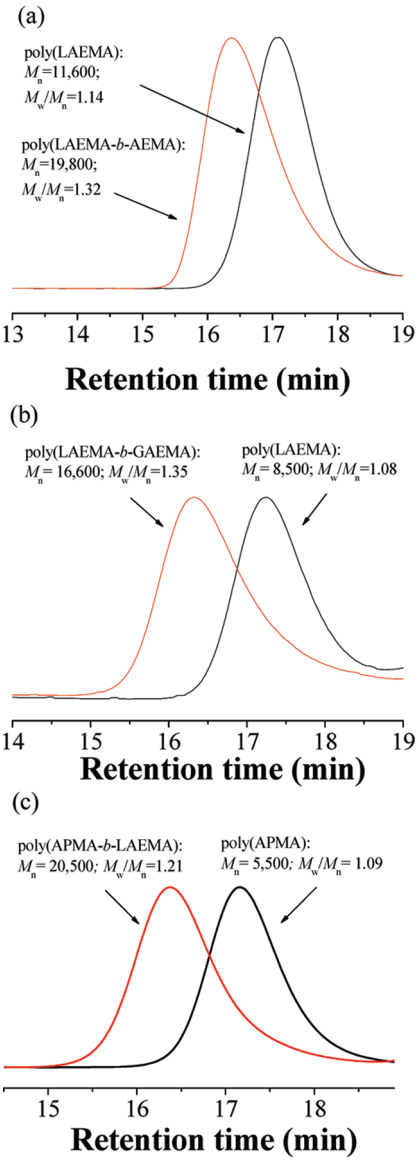


Figure 6. Aqueous gel permeation chromatography traces for the blocking experiment showing the macro-CTA and resulting copolymers. Key: (a) poly(LAEMA) macro-CTA and the resulting polymer poly(LAEMA-*b*-AEMA) (monomer, 0.10 g; macro-CTA, 0.10 g; ACVA, 0.001 g; solvent, H_2O/DMF, 9:1 v/v); (b) poly(LAEMA) macro-CTA and the resulting polymer poly(LAEMA-*b*-GAEMA) (monomer, 0.10 g; macro-CTA, 0.10 g; ACVA, 0.001 g; solvent, H_2O/DMF, 9:1 v/v); (c) poly(APMA) macro-CTA and the resulting polymer poly(APMA-*b*-LAEMA) (monomer, 1.60 g; macro-CTA, 0.48 g; ACVA, 0.009 g; solvent, H_2O/DMF, 8:0.5 v/v).

does not provide a precise quantification of the number of biotin groups. Despite the fact that the yield needs to be improved, "hetero-biofunctional macro-crosslinker" was successfully synthesized containing approximate 9 biotin and 32 galactose moieties per polymer chain.

Gold Nanoparticles Synthesis and Lectin Recognition. On the basis of the glycocluster effects,[34] carbohydrates functionalized gold nanoparticles can amplify the effect of carbohydrate-lectin interaction. Previously, α-lLactosyl-ω-mercaptopoly(ethylene glycol) modified gold nanoparticles were employed in a study of lection recognition by Otsuka et al.[53] Herein, different types of glyco-copolymers were used as stabilizers to synthesize gold nanoparticles (AuNPs) and those gold nanoparticles were subsequently used in the studies of carbohydrate-lectin interactions. Gold nanoparticles

were selected not only due to their excellent optical properties and ease of functionalization, but also due to their low cytotoxicity, biocompatibility and excellent colloidal stability. The gold nanoparticles used in this study were synthesized according to Brust et al.'s work.[42] In the presence of sodium borohydride, Au^{3+} was reduced to Au^0 and the dithio ester end groups of RAFT synthesized polymers were reduced *in situ* to stabilize the formed gold nanoparticles. These polymers were attached to the surface of gold nanoparticles through the soft−soft interaction between gold and sulfur. It should be noted that primary amine moieties can also take part in the stabilization.[54] The results of the surface functionalization of the gold nanoparticles with the synthesized polymers are shown in Table 5. The synthesized glyconanoparticles are fairly monodisperse and exhibited long-term colloidal stability (weeks) in aqueous solution. Next, our motivation is to establish whether or not the carbohydrate and biotin groups on the nanoparticle surface are available for biomolecular recognition processes.

RCA_{120} from castor bean is one of the most widely used lectins for recognizing galactose/lactose residues. Thus, RCA_{120} was first used to investigate the lectin-binding activity of poly(BAPMA-*b*-LAEMA). Aliquot of poly(BAPMA-*b*-LAEMA) stabilized AuNPs solution (AuNP-1) were diluted in PBS. After the addition of RCA_{120} solution, the mixture (RCA_{120} concentration, 0.6 mg/mL; LAEMA concentration, 7.7 nmol/mL) was analyzed by dynamic light scattering (DLS) and UV−visible spectroscopy. The DLS intensity distribution result showed that gold nanoparticles size increased from 52 to 362 nm (Table 5, AuNP-1). Due to the possibility of lectin self-aggregation, UV−visible spectroscopy was employed to examine the aggregation. After 3 h incubation, an obvious decrease of UV absorbance was noted, which indicated a reduction of the solutes concentration *via* precipitation (Figure 10). The red color of flocculates can be seen clearly, which further confirmed that the precipitates were poly(BAPMA-*b*-LAEMA) stabilized AuNPs, not only the lectins (Figure S6).

Similar to biotin, HABA is a dye with binding affinity to avidin. In the presence of biotin or biotinylated reagents, HABA is displaced quantitatively by available biotin due to the higher affinity of biotin ($K_d = 10^{-15}$ M for biotin against $K_d = 10^{-6}$ M for HABA). As shown in Figure 9, the decrease of UV absorbance after the addition of biotinylated gold nanoparticles to HABA/avidin solution indicated the availability of the biotin groups on the surface of the gold nanoparticles for conjugation. Moreover, biotin concentration (102.6 nmol/mL) of the gold nanoparticles solution can be further quantified. Subsequently, streptavidin was also employed to examine the activity of biotin moieties on poly(BAPMA-*b*-LAEMA). After the addition of streptavidin solution, a decrease of absorbance was noted after 24 h (Figure 11). Both the HABA/avidin assay and streptavidin recognition indicated the availability of biotin moieties toward specific biomolecular recognition processes. The ability of cross-linking different types of biomolecules was examined using the DotLab system. The DotLab system is a relatively new optical biosensor based on diffractive optics technology. This system uses a polystyrene based sensor chip with an integrated prism situated below the flow channel, which can monitors biomolecule binding in real time and in complex media. Because diffraction is inherently self-referencing, nonspecific binding to both the patterned and nonpatterned regions will not affect the signal. Such characteristic offers an important advantage over other optical biosensor systems in which any surface binding event will cause an increase in signal.[55] A change of response was observed after the injection of poly(BAPMA-*b*-LAEMA) stabilized gold nanoparticles (AuNP-1), which indicated the binding of biotin moiety on poly(BAPMA-*b*-LAEMA) stabilized gold nanoparticles to avidin on the sensor chip. After washing away the nonbinding gold nanoparticles, the sensor chip should be theoretically coated with poly(BAPMA-*b*-LAEMA). Therefore, RCA_{120} solution was subsequently injected into the system. A higher response was noted and confirmed the further binding of galactose moieties on poly(BAPMA-*b*-LAEMA) to RCA_{120} lectin (Figure 12).

Jacalin lectin was also used in the studies of carbohydrate-lectin interaction. Jacalin is extracted from Jack fruit seeds; a glycoprotein reported to bind human serum and secretory IgA.[56] The lectin is specific toward the Thomsen−Friedenreich (T) antigen and Jacalin was also demonstrated to be a mitogen of T-lymphocytes.[57] After the addition of Jacalin, DLS intensity distribution result showed that the size of poly(LAEMA-*b*-GAEMA) stabilized gold nanoparticles

Table 3. Summary of Synthetic Parameters for the Reversible Addition−Fragmentation Chain Transfer Copolymerization of 2-Lactobionamidoethyl Methacrylamide

	homopolymer		copolymer	
copolymer type	M_n (g/mol)	M_w/M_n	M_n (g/mol)	M_w/M_n
poly($LAEMA_{25}$-*b*-$AEMA_{50}$)	11600	1.14	19800	1.32
poly($LAEMA_{18}$-*b*-$GAEMA_{27}$)	8500	1.08	16600	1.35
poly($APMA_{31}$-*b*-$LAEMA_{32}$)	5500	1.09	20500	1.21

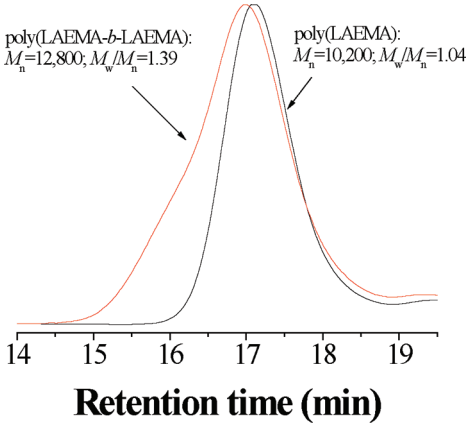


Figure 7. Aqueous gel permeation chromatography traces for the self-blocking experiment showing the poly(LAEMA) macro-CTA and resulting polymer (reaction time of macro-CTA synthesis, 15 h; initial mole ratio of CTA to initiator, 3; Table 4, entry 3).

Table 4. Summary of Synthetic Parameters for Poly(LAEMA) Self-Blocking Experiments in Different Reaction Conditions

	macro-CTA				self-blocking polymer	
entry	[CTP]/[ACVA]	reaction time (h)	M_n (g/mol)	M_w/M_n	M_n (g/mol)	M_w/M_n
1	3	4	8600	1.12	15000	1.24
2	0.5	3	8200	1.10	11400	1.25
3	3	15	10200	1.04	12800	1.39

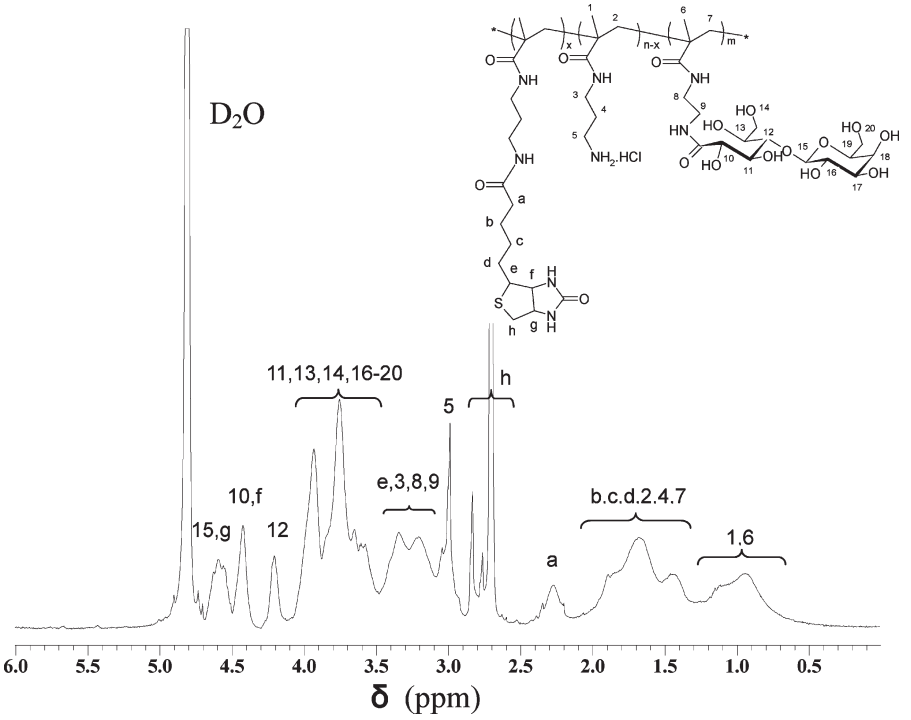


Figure 8. ^{1}H NMR (D_2O) spectrum of poly(BAPMA-*b*-LAEMA).

Scheme 4. Modification of Poly(APMA-*b*-LAEMA) with Biotinyl-*N*-hydroxysuccinimide Ester

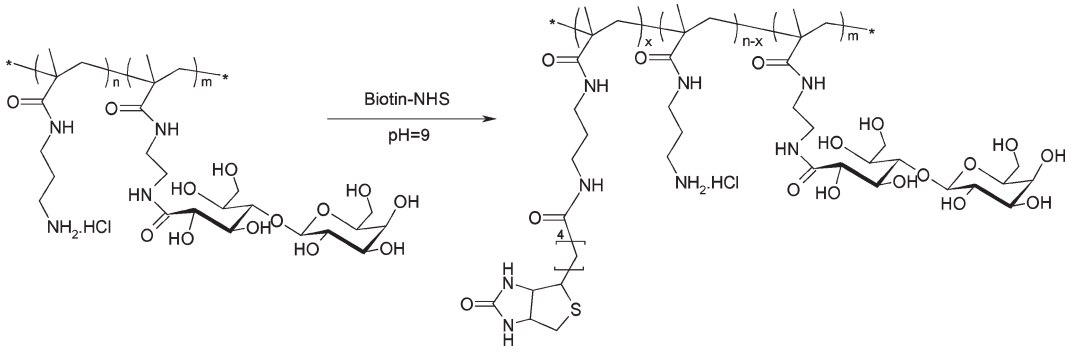


Table 5. Dynamic Light Scattering Data of Gold Nanoparticles Solution

		AuNPs			AuNPs + lectin	
code	stabilizer	R_h (nm)	% RSD	lectin	R_h (nm)	% RSD
AuNP-1	poly(BAPMA-*b*-LAEMA)	52.18	19.4	RCA_{120}	361.95	15.3
AuNP-2	poly(APMA-*b*-LAEMA)	40.2	24.7	ConA	50.4	27.8
AuNP-3	poly(LAEMA-*b*-GAEMA)	29.5	33.9	Jacalin	23.84	26.3
					387.83	26.7
AuNP-4	poly(LAEMA-*b*-GAEMA)	26.52	14.4	ConA	47.61	18.0
					155.13	12.7

increased from 30 to 388 nm (Table 5, AuNP-3). Further studies related to Jacalin and galactose-containing chain segment were conducted using UV−visible spectroscopy. UV absorbance of the solution containing 0.5 mg/mL Jacalin lectin and AuNP-2 gold nanoparticles solution (LAEMA concentration 47.3 nmol/mL, APMA concentration 45.8 nmol/mL) was recorded. A decrease in absorbance was found over time (Figure 13a). As control experiments, the combination of Jacalin lectin (0.5 mg/mL) and AuNP-3 gold nanoparticles solution (LAEMA concentration

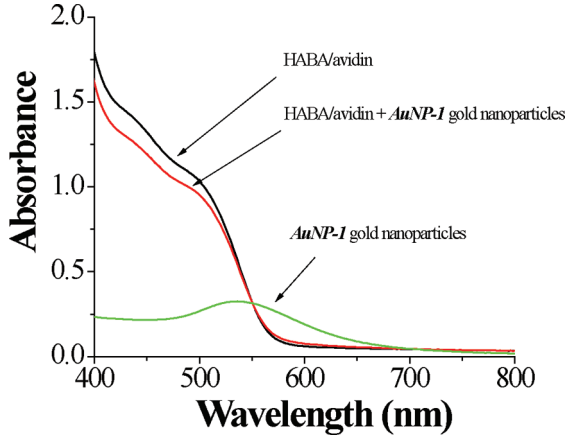


Figure 9. UV−visible spectrum for 4-hydroxyazobenzene 2-carboxylic acid (HABA)/avidin test of poly(BAPMA-*b*-LAEMA) stabilized gold nanoparticles (AuNP-1).

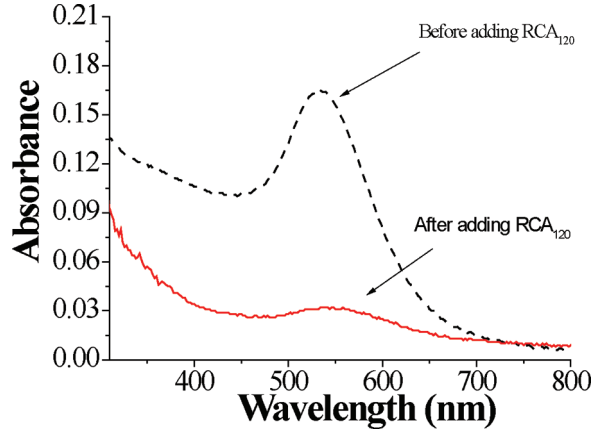


Figure 10. UV−visible spectrum of poly(BAPMA-*b*-LAEMA) stabilized gold nanoparticles (AuNP-1) phosphate buffer solution (pH = 7.4; RCA_{120} concentration = 0.6 mg/mL; LAEMA concentration = 7.7 nmol/mL) before (dash line) and 3 h after the addition of *R. communis* agglutinin (solid line).

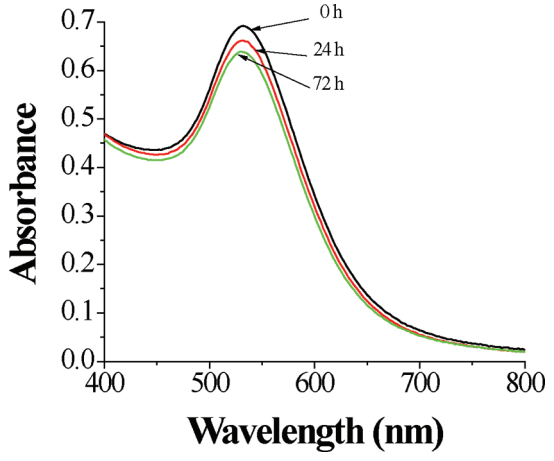


Figure 11. Evolution of AuNPs' UV absorbance in pH = 7.4 phosphate buffer solution with time. Poly(BAPMA-*b*-LAEMA) stabilized gold nanoparticles (AuNP-1) with 0.3 mg/mL streptavidin; biotin concentration = 9.8 nmol/mL.

26.3 nmol/mL, GAEMA concentration 39.4 nmol/mL) as well as the combination of Con A (Type IV, from *Canavalia ensiformis* (Jack bean), 0.5 mg/mL) and AuNP-2 gold

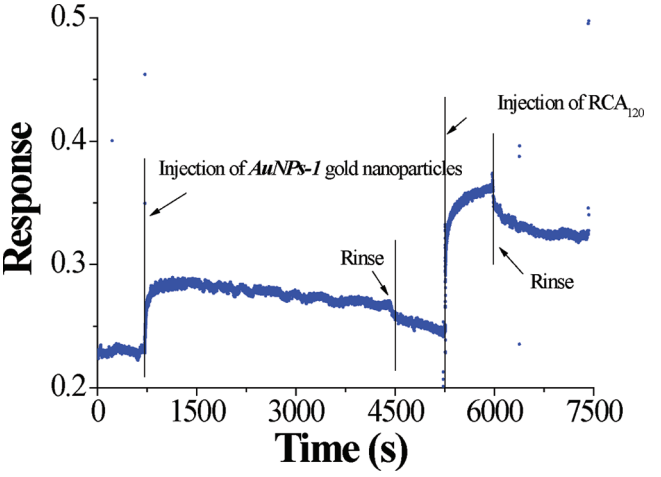


Figure 12. DotLab sensorgram for the bioconjugation of the poly(BAPMA-*b*-LAEMA) stabilized gold nanoparticles (AuNP-1) to avidin coated sensor chip and the bioconjugation to *R. communis* agglutinin.

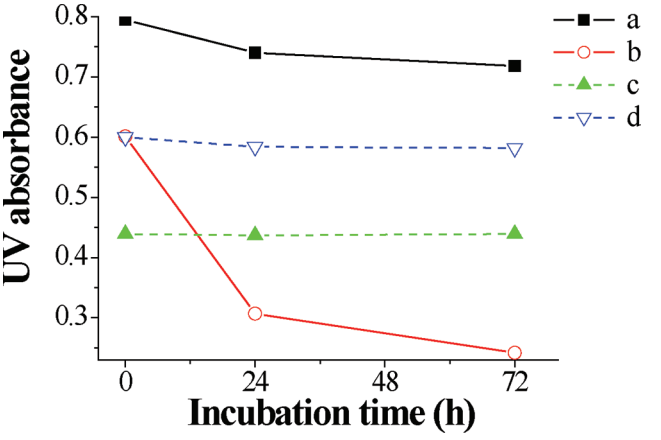


Figure 13. Maximum UV absorbance at 520 nm wavelength of gold nanoparticles in phosphate buffer solution (pH = 7, 1.0×10^{-4} mmol/mL Ca^{2+}, 1.0×10^{-4} mmol/mL Mn^{2+}) plots against time. Gold nanoparticles with cationic polymer chain segment (bold symbols) and with linear glucose chain segment (open symbols). In the presence of 0.5 mg/mL Jacalin (solid lines) or Concanavalin A (dash lines). Stabilized by poly(APMA-*b*-LAEMA) (Entry AuNP-2), LAEMA concentration 47.3 nmol/mL, APMA concentration 45.8 nmol/mL (a, c). Stabilized by poly(LAEMA-*b*-GAEMA) (Entry AuNP-3), LAEMA concentration 26.3 nmol/mL, GAEMA concentration 39.4 nmol/mL) (b). Stabilized by poly(LAEMA-*b*-GAEMA) (Entry AuNP-4), LAEMA concentration 26.3 nmol/mL, GAEMA concentration 39.4 nmol/mL) (d).

nanoparticles solution were examined by UV−visible spectroscopy. A decrease of UV absorbance was found in all cases when Jacalin lectin involved (Figure 13a and Figure 13b), while no change was noticed in the presence of Con A (Figure 13c).

The results mentioned above indicated that LAEMA (galactose-containing) chain segments have specific affinity to RCA_{120} and Jacalin lectins but not to Con A lectin.

Two previous reports mentioned about the possible Con A recognition ability of GAMA components containing the same carbohydrate moiety as GAEMA (Supporting Information Scheme S1).[58] Although the DLS results indicated an increase of particles size (from 27 to 155 nm) after the addition of Con A to poly(LAEMA-*b*-GAEMA) stabilized gold nanoparticles (Table 5, AuNP-4), the possibility of the self-aggregation of Con A cannot be eliminated. The

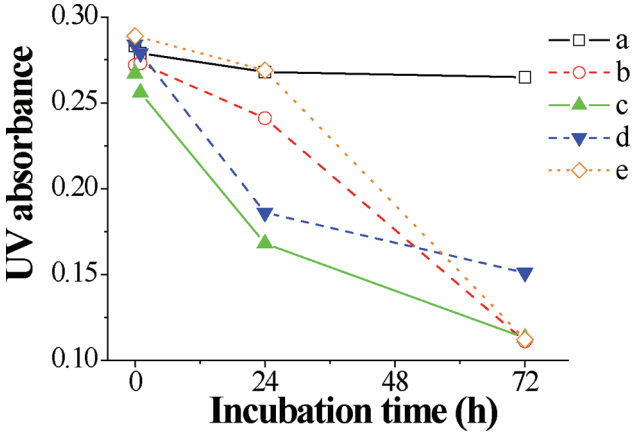


Figure 14. Maximum UV absorbance around 520 nm wavelength of gold nanoparticles in phosphate buffer solution (pH = 7, 2.5×10^{-2} mmol/mL Ca^{2+}, 2.5×10^{-2} mmol/mL Mn^{2+}) plots against time: gold nanoparticles with cationic polymer chain segment (bold symbols) and with linear glucose chain segment (open symbols); without protein (solid lines); in the presence of 0.5 mg/mL Concanavalin A (dashed lines) or bovine serum albumin (dotted line). Key: stabilized by poly(LAEMA-*b*-GAEMA) (entry AuNP-4), LAEMA concentration 26.3 nmol/mL, GAEMA concentration 39.4 nmol/mL (a, b, e); stabilized by poly(APMA-*b*-LAEMA) (entry AuNP-2), LAEMA concentration 47.3 nmol/mL, APMA concentration 45.8 nmol/mL (c, d).

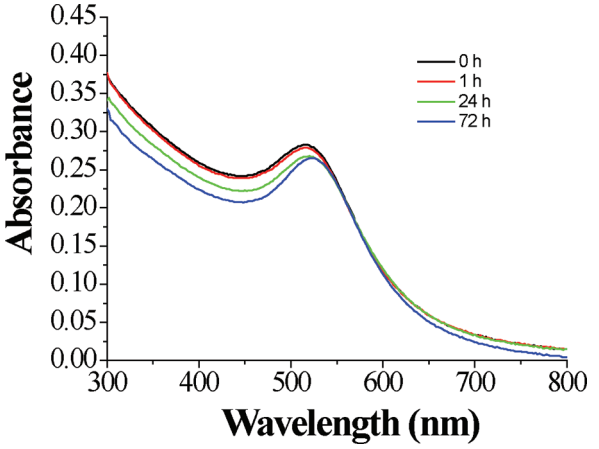


Figure 15. Evolution of UV absorbance of gold nanoparticles in phosphate buffer solution (pH = 7, 2.5×10^{-2} mmol/mL Ca^{2+}, 2.5×10^{-2} mmol/mL Mn^{2+}) with time. Poly(LAEMA-*b*-GAEMA) stabilized gold nanoparticles (AuNP-4): LAEMA concentration 26.3 nmol/mL; GAEMA concentration 39.4 nmol/mL. Without protein.

gold nanoparticles used in this study have a characteristic UV absorption around 520 nm wavelength, therefore UV−visible spectroscopy was used in further studies. In our case, when Con A (Type IV, from *C. ensiformis* (Jack bean)) involved, only a slight decrease (~0.016) of UV absorbance was found in the present of 1.0×10^{-4} mmol/mL Ca^{2+} and Mn^{2+} salts (Figure 13d). In order to eliminate the effect of gold nanoparticles concentration, similar tests were also conducted in different gold nanoparticles concentrations. A slight decrease (~0.016) of UV absorbance was observed in all cases after 72 h (See Supporting Information, Figure S7). Considering the active function of Ca^{2+} and Mn^{2+} salts in recognition process,[59] several experiments related to the salts concentration were conducted with poly(LAEMA-*b*-GAEMA) stabilized gold nanoparticles (AuNP-4) and poly(APMA-*b*-LAEMA) stabilized gold nanoparticles (AuNP-2). All the experiments were conducted at low pH (pH ≤ 7.0) to prevent the self-aggregation of Con A. In the presence of 2.5×10^{-2} mmol/mL Ca^{2+} and 2.5×10^{-2} mmol/mL Mn^{2+}, a decrease of UV absorbance was found in all cases. More obvious decrease in absorbance was noticed after 72 h in the sample with Con A (Figure 14b against Figure 14a). Moreover, compared to the tests with 1.0×10^{-4} mmol/mL Ca^{2+} and Mn^{2+} salts, this decrease in absorbance was found 10 times larger (~0.16 against ~0.016). However, obvious decreases in absorbance were also seen in poly(APMA-*b*-LAEMA) stabilized gold nanoparticles (AuNP-2) with a similar decreasing trend, no matter if it involved Con A (Figure 14, parts c and d), which may be due to the cationic primary amine pendant groups of poly(APMA-*b*-LAEMA). As part of the control experiment, BSA was employed to study the nonspecific adsorption. Unfortunately, a similar decrease of UV absorbance was still observed (Figure 14e against Figure 14b). We believed that the interaction between GAEMA moiety and Con A was nonspecific adsorption and such result was also found coincident with Yang et al.'s work.[60] From Figure 15, even without any protein, a decrease of UV absorbance as well as a slight red shift of UV spectra were found. The aggregation of gold nanoparticles may be due to the Ca^{2+} mediated carbohydrate-carbohydrate interactions.[61] Accordingly, the carbohydrate-lectin interaction may be under a similar mechanism in the presence of 2.5×10^{-2} mmol/mL Ca^{2+} and 2.5×10^{-2} mmol/mL Mn^{2+}. Although the final result showed no specific Con A recognition ability of GAEMA moiety, some other interesting results was still found: first, nonspecific carbohydrate−protein adsorption increases with salt concentration (Figure 13d and 14b); second, with the same anion (Cl^-), the involvement of cationic chain segment can enhance the aggregation of glycopolymer (Figure 14, parts a and c).

Conclusion

In this work, a novel galactose-containing monomer LAEMA was synthesized from lactobionic acid without employing any protecting group chemistry and polymerized *via* RAFT process in a control manner. Chain extension ability of RAFT synthesized poly(LAEMA) was examined and successfully employed as macro-CTA to synthesize copolymers containing various pendant groups for specific bioconjugation. Primary amine pendant groups of poly(APMA-*b*-LAEMA) were further modified with biotin−NHS in ~30% yield. Different kinds of galactose-containing copolymers were used as stabilizers to synthesize gold nanoparticles. Lectin recognition between poly(APMA-*b*-LAEMA), poly(LAEMA-*b*-GAEMA), poly(BAPMA-*b*-LAEMA) and RCA_{120}, Con A, Jacalin were investigated by means of monitoring changes in turbidity of AuNPs solution. Interaction between LAEMA (galactose-containing) segment and RCA_{120} or Jacalin was observed. DotLab sensorgram showed biomolecular recognition event between the biotinylated glyconanoparticles with both avidin and RCA_{120}, indicating the availability of biotin and galactose residues on the nanoparticle surface toward bioconjugation. Such biomaterials may act as macro heterobifunctional cross-linkers with higher efficiency in biomedical applications. Nonspecific protein adsorption of poly(LAEMA-*b*-GAEMA) stabilized gold nanoparticles was found to increase with increasing salt concentration. In the presence of 2.5×10^{-2} mmol/mL Ca^{2+} and Mn^{2+} salts, even with the same anion (Cl^-), the involvement of cationic chain segment enhanced the aggregation of glycopolymer.

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and Knowledge), and Axela Biosensors Inc. for financial support of this work.

Supporting Information Available: Scheme showing the chemical structures of LAMA and GAMA, figures showing the mass spectrum for the LAEMA monomer, tables of synthetic parameters figures showing ^{1}H NMR spectra of the LAEMA homopolymer and diblock copolymer, LAMA polymer, a kinetic data of the LAEMA RAFT homopolymerization with target DP_n of 27 GPC trace for the LAEMA self-blocking experiments, UV−vis spectra of gold nanoparticles for the lectin recognition experiments, images of gold nanoparticles incubated with lectins, and DotLab plot. This material is available free of charge via the Internet at http://pubs.acs.org.

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