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Preparation of Biotinylated Glyconanoparticles via a Photochemical Process and Study of Their Bioconjugation to Streptavidin

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We report here the preparation of novel biotinylated glyconanoparticles from well-defined biotinylated glycopolymers and poly(*N*-isopropylacrylamide) (PNIPAAm) synthesized via the reversible addition fragmentation chain transfer (RAFT) polymerization process. The in situ reduction of the biotinylated glycopolymers, PNIPAAm, poly(ethylene glycol), and $HAuCl_4$ via a photochemical process resulted in the formation of biotinylated gold nanoparticles. The multifunctional biotinylated glyconanoparticles were then evaluated for their bioconjugation toward streptavidin using UV−vis spectroscopy and surface plasmon resonance (SPR). The biotinylated nanoparticles underwent aggregation in the presence of streptavidin as revealed by spectrophotometry, which indicates the accessibility of the biotin for conjugation. These results were further confirmed by surface plasmon resonance even in the case of surface-immobilized streptavidin.

Introduction

Gold nanoparticles are receiving an enormous amount of attention because of their unique applications in many fields such as biomedicine, bionanotechnology, optics, and electronics.[1,2] Thiol chemistry has been widely used for the surface modification of gold nanoparticles with chemical species ranging from small molecules to synthetic and natural polymers being reported.[3−17] The surface engineering of gold nanoparticles plays an essential role in improving their biocompatibility and colloidal stability and hence in imparting desirable size-dependent electronic, magnetic, and optical properties, as well as enabling conjugation of bioactive functional groups.[3]

Most biomedical applications requiring gold nanoparticles rely on the use of poly(ethylene glycol) stabilized (PEGylated) gold nanoparticles.[18] The hydrophilic PEG induces the easy aqueous dispersion of the gold colloids and also helps to prevent nonspecific interactions. Gold glyconanoparticles have recently become the focus of intense interest in the biomedical field partly because of the increasing understanding of the biological recognition phenomena involving carbohydrates and proteins.[19−31] The presence of the carbohydrate moieties on the nanoparticle surface has proven to have a drastic effect on the biomolecular

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events. For example, certain carbohydrate moieties, such as galactose residues, exhibit high recognition toward hepatocytes.[32] Those specific carbohydrate residues can be easily attached to the nanoparticle surface for targeted delivery. Furthermore, highly monodisperse and multivalent gold glyconanoparticles showed promise as highly stable and biocompatible materials that can be easily synthesized in an aqueous environment.

With the advent of living radical polymerization techniques (LRP) such as atom transfer radical polymerization (ATRP),[33] and reversible addition fragmentation chain transfer polymerization (RAFT),[34] glycopolymers of controlled dimensions (narrow polydispersities and predetermined molecular weights) have been prepared with or without protection of the hydroxyl groups.[35−45] Temperature-responsive poly(*N*-isopropylacrylamide) (PNIPAAm) has also been successfully prepared by the RAFT polymerization methods.[46−60] The use of well-defined PNIPAAm as stabilizing agents for gold nanoparticles has also been well-investigated.[61−65]

In this work, we describe the synthesis of well-defined biotinylated glycopolymers and PNIPAAm via the RAFT process. Multifunctional glyconanoparticles were then prepared for the first time by the in situ photochemical reduction of the RAFT-prepared glycopolymers, PNIPAAm, poly(ethylene glycol), and gold tetrachloride ($HAuCl_4 \cdot 3H_2O$). The biotinylated glyconanoparticles were then evaluated for their ability to bind with high affinity to streptavidin. As expected, the biotinylated gold nanoparticles underwent particle aggregation indicating the accessibility of the biotin for bioconjugation to streptavidin. No aggregation of the glyconanoparticles was noted after weeks that indicated a very high colloidal stability of those nanoparticles.

Experimental Section

Materials. All chemicals were purchased from Sigma-Aldrich, Anachemia, or Acros Chemicals. *N*-Isopropylacrylamide (NIPAAm) was recrystallized in hexane before use. *N*-Acryloylmorpholine (NAM) (Aldrich, 97%) was distilled under reduced pressure (120 °C, 10 mmHg). 2,2′-Azobis(isobutyronitrile) (AIBN) (Fluka, 98%) was purified by recrystallization from ethanol. 1,4-Dioxane (Acros, 99%) was distilled over $LiAlH_4$ (110 °C). Trioxane (Acros, 99%) and other materials were used without further purification. Synthesis of the RAFT agents, *tert*-butyl dithiobenzoate (*t*BDB)[66] and biotin derivative (biotin-CTA),[67] has been described previously. All other chemicals were used as received. The galactose derivative, 6-*O*-acrylamido-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose was synthesized as described previously.[68] The photoinitiator, Irgacure-2959, was donated by CIBA Speciality Chemicals.

Characterization. *PNIPAAm GPC Analysis.* Molecular weights and molecular weight distributions were assessed by gel permeation chromatography (GPC) using an organic eluent. The following protocols were used in this work. The polymers were analyzed using a Viscotek GPC instrument. The system is comprosed of a dual detector (refractive index detector and viscometer) and mixed Viscogel columns, and DMF containing 0.1 M LiBr was used as eluent at a flow rate of 1.0 $mL.min^{-1}$. Calibration was achieved using a series of monodisperse polystyrene standards. The molecular weight and polydispersity data were calculated using the *OmniSEC* software package.

Glycopolymer GPC Analysis. Molecular weight distributions of the protected glycopolymers were determined by GPC in THF (SDS, 99%), using a Waters column (Styragel HR4E). The flow rate was maintained at 1 $mL.min^{-1}$ using a Waters 1515 isocratic HPLC pump. Analyzes were performed by injection of 20 μL of polymer solution (10 $mg.mL^{-1}$) in THF. Detection was performed using a Waters 2410 differential refractometer. The molecular weight and polydispersity data were determined using the Waters *Breeze* software package, according to a polystyrene calibration.

In the case of the deprotected glycopolymers, molecular weight distributions were determined by aqueous size exclusion chromatography (SEC) coupled to a light scattering detection (LSD), using a Waters 510 pump and two Waters Ultrahydrogel columns (2000 and 500 Å). On-line double detection was provided by a differential refractometer (DRI Waters 410) and a three-angle (47°, 90°, 130°) MiniDAWN light scattering photometer (Wyatt Technologies), operating at 690 nm. Analyses were performed by injection of 200 μL of polymer solution (5 $mg.mL^{-1}$) in a borate buffer (pH = 9.3, 0.05 M), previously filtered through a 0.22 μm Millipore filter and used as eluent at a flow rate of 0.5 $mL.min^{-1}$ (35 °C). The specific refractive index increment (d*n*/d*c*) for poly(NAM) in the same eluent (0.163) was previously determined with a NFT ScanRef monocolor interferometer operating at 633 nm. The molecular weight and polydispersity data were determined using the Wyatt *ASTRA SEC/LS* software package.

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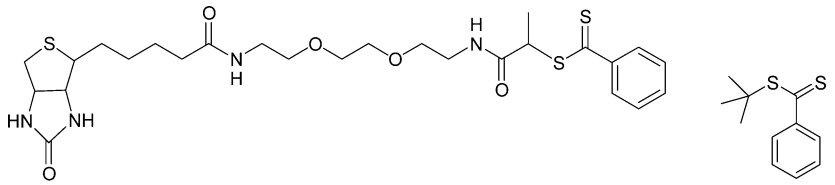

Figure 1. Structure of the chain transfer agents: biotin-labeled chain transfer agent (biotin-CTA)[67] and *t*BDB[66].

Scheme 1. Synthesis and Deprotection of Glycopolymers

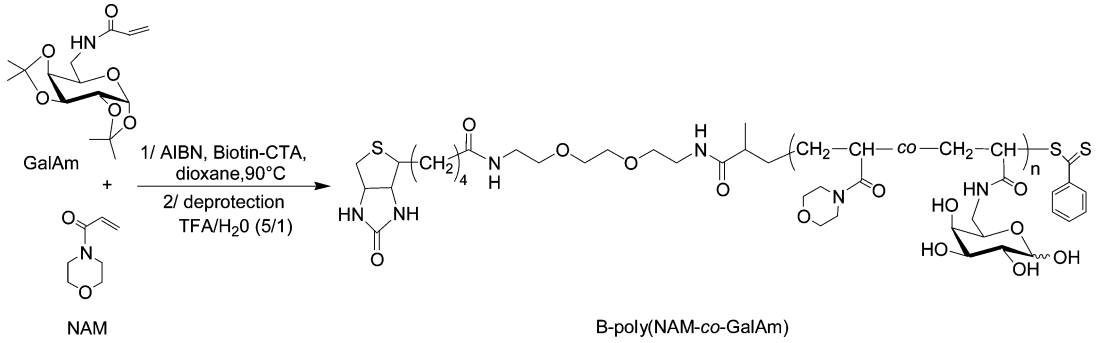

Table 1. Synthetic Parameters for the Synthesis of the Biotinylated and Non-Biotinylated Glycopolymers

sample (α-end group)	global conversion (%)	calculated M_n[a] (deprotected form) (g.mol^{-1})	$M_{n\ exp.}$ (g.mol^{-1})	M_w/M_n	nb. NAM/nb. GalAm per chain
B-poly(NAM-*co*-GalAm)-1	33	2000	2306[b]	1.15[b]	7/2
B-poly(NAM-*co*-GalAm)-2	79	43 900	n.d.[c]	n.d.[c]	224/50
poly(NAM-*co*-GalAm)	42	22 600	24 400[d]	1.05[d]	127/20

[a] Calculated $M_n = ([NAM]_0 \times M_{NAM} \times C_{NAM} + [GalAm]_0 \times M_{GalAm\ deprotected} \times C_{GalAm})/[ATC]_0 + M_{Biotin-CTA}$. [b] Determined by MALDI-TOF MS on the protected glycopolymer. [c] It was not possible to determine the M_n by SEC/RI/LS due to filtration problem (0.22 μm) of the sample (possible presence of aggregates). [d] Determined by SEC/RI/LS in aqueous phase.

The matrix-assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-ToF MS) analyses were performed on a Voyager-DE STR (Applied Biosystems, Foster City, CA).

H and ^{13}C NMR spectra were recorded on a 200 MHz Varian spectrometer. Cloud points of PNIPAAm and mixtures were obtained using a thermoregulated Cary 100 Bio UV−visible spectrophotometer coupled to a temperature controller in the absorbance mode at a wavelength of 380 nm and a heating rate of 0.5 °C/min. Spectra of dilute aqueous gold dispersions were also recorded using a Biochrom UV−vis spectrophotometer.

Glyconanoparticles Analysis. A model 802 Viscotek DLS instrument operating at a laser wavelength of 825−832 nm was used for particle size measurements in highly dilute aqueous gold dispersions.

Surface Plasmon Resonance Measurements. A Biacore X SPR was used to study the binding events between the biotinylated gold nanoparticles and the surface-immobilized streptavidin sensor chip. The buffer (HEPES-0.01 M, 0.15 M NaCl, 0.005% surfactant P20) and SA sensor chip were purchased from Biacore Inc. The flow rate was set to 10 or 20 μL.min^{-1}, and between 25 and 50 μL of sample was injected at a time.

Synthesis of *S*,*S*′-Bis (α,α′-dimethyl-α″acetic acid)trithiocarbonate (CTA$_1$). The synthesis of CTA$_1$ was conducted as previously reported.[69] Carbon disulfide (27.4 g, 0.36 mol), chloroform (107.5 g, 0.9 mol), acetone (52.3 g, 0.9 mol), and tetrabutylammonium hydrogen sulfate (2.41 g, 7.1 mmol) were mixed with 120 mL of toluene/benzene 1/1 mixture in a 1 L jacketed reactor cooled with tap water under nitrogen. Sodium hydroxide (50%) (201.6 g, 2.52 mol) was added dropwise over 300 min in order to keep the temperature below 25 °C. The reaction was stirred overnight. 900 mL of water was then added to dissolve the solid, followed by 120 mL of concentrated HCl (*caution! gas evolved with a mercaptan odor*) to acidify the aqueous layer. The mixture was stirred for 30 min under nitrogen. The solid was filtered and rinsed thoroughly with water. The product was dried to constant weight to collect 26 g of reddish brown solid. It was further purified by stirring in toluene/acetone (4/1) or by recrystallizations from 60% 2-propanol or acetone to afford a yellow crystalline solid; mp 178 °C.

^{1}H NMR (DMSO-d_6, ppm): 1.28 (s, 12H), 12.20 (s, 2H). ^{13}C NMR (DMSO-d_6,ppm): 25.53, 56.74, 173.51, 219.40.

Typical Synthesis of PNIPAAm by the RAFT Process. *N*-Isopropylacrylamide (2.0 g, 18 mmol, target DP$_n$ = 50) was dissolved in 5 mL of degassed dioxane. 4,4′-Azobis(4-cyanovaleric acid) (12 mg, 43 μmol) and *S*,*S*′-bis(α,α′-dimethyl-α″-acetic acid)-trithiocarbonate (CTA$_1$) (58 mg, 0.2 mmol) were added, and the solution was then purged with nitrogen. The tube was sealed, and the polymerization reaction was carried out at a temperature of 60 °C for 24 h. The viscous polymer solution was cooled to room temperature and then diluted with distilled deionized water. The polymer solution was dialyzed for 48 h using a dialysis membrane of molecular weight cutoff (MWCO) of 3400. The polymer was then freeze-dried overnight.

RAFT Polymerization of the Galactose Derivative (GalAm). RAFT polymerization of GalAm in the presence of biotin-CTA has been recently reported.[68] A typical procedure for the RAFT polymerization of GalAm is described below.

NAM (0.431 g, 3.05 mmol), GalAm (0.239 g, 0.76 mmol), biotin-CTA (0.089 g, 0.15 mmol), AIBN (2.5 mg, 0.015 mmol), dioxane (2 mL), and trioxane (0.029 g, internal reference for ^{1}H NMR determination of monomer consumption) were introduced in a Schlenk tube equipped with a magnetic stirrer. The mixture was degassed by five freeze−evacuate−thaw cycles and then heated under nitrogen in a thermostated oil bath. Periodically, samples were withdrawn from the polymerization medium for analyses. Similar experimental conditions were used with *t*BDB instead of biotin-CTA. Monomer conversion was determined by ^{1}H NMR spectroscopy

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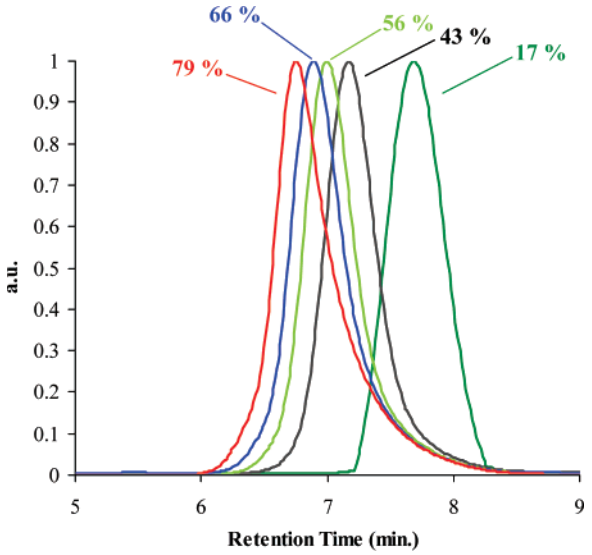

Figure 2. GPC chromatograms (THF/PS standards) of protected biotinylated glycopolymers (sample at 79% is B-poly(NAM-*co*-GalAm)-2).

Scheme 2. Raft Polymerization of *N*-Isopropylacrylamide (NIPAAm)

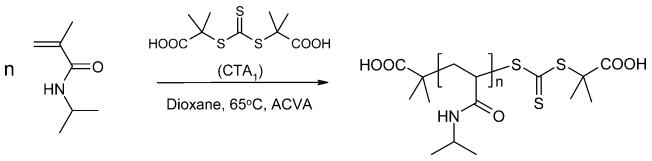

Table 2. Synthetic Parameters for the RAFT Polymerization of NIPAAm in Dioxane at 65 °C

samples	chain transfer agent	CTA[a]-initiator ratio	final yield[c] (%)	M_n[b]	M_w/M_n
PNIPAAm-1	CTA_1	5	-	6540	1.06
PNIPAAm-2	CTA_1	10	60	6201	1.10
PNIPAAm-3	CTA_1	10	55	15 000	1.20
PNIPAAm-4	CTA_1	10	77	19 169	1.24

[a] Chain transfer agent. [b] Determined by GPC using DMF as eluent containing 0.1 M LiBr and polystyrene as calibration standards. [c] The conversions were determined by ^{1}H NMR and were found to be higher than 95%. However, after purification, the yields of the PNIPAAm homopolymers were found to be between 60% and 77%.

using a Bruker AC 200 spectrometer (200 MHz), by comparison of the vinylic protons of NAM (5.72 ppm) and of GalAm (5.61 ppm) with trioxane (5.15 ppm) used as internal reference.[70] Typically, 400 μL of *d*-chloroform was added to 200 μL of each sample. Similar experimental conditions were used with *t*BDB.

Polymer samples were precipitated in a large volume of diethyl ether, recovered by centrifugation, and finally dried under air before analysis by SEC.

Deprotection of the Glycopolymer Samples. Typically, for polymer sample B-poly(NAM-*co*-GalAm)-2 (0.054 g, 1.13×10^{-6} mol) was dissolved in a water/trifluoroacetic acid (TFA) (1/5 v/v) mixture (200 μL). The solution was left under stirring condition at room temperature for 4 h. After evaporation of the excess of TFA and recovered acetone, the samples were dried under vacuum.

Formation of Gold Glyconanoparticles (AuNPs). The following protocol is carried out for the synthesis of gold glyconanoparticles. The functionalized nanoparticles contain a mixture of PNIPAAm, MPEG-SH, and the biotinylated glycopolymer.

(70) D'Agosto, F.; Charreyre, M.-T.; Véron, L.; Llauro, M.-F.; Pichot, C. *Macromol. Chem. Phys.* **2001**, *202*, 1689−1699.

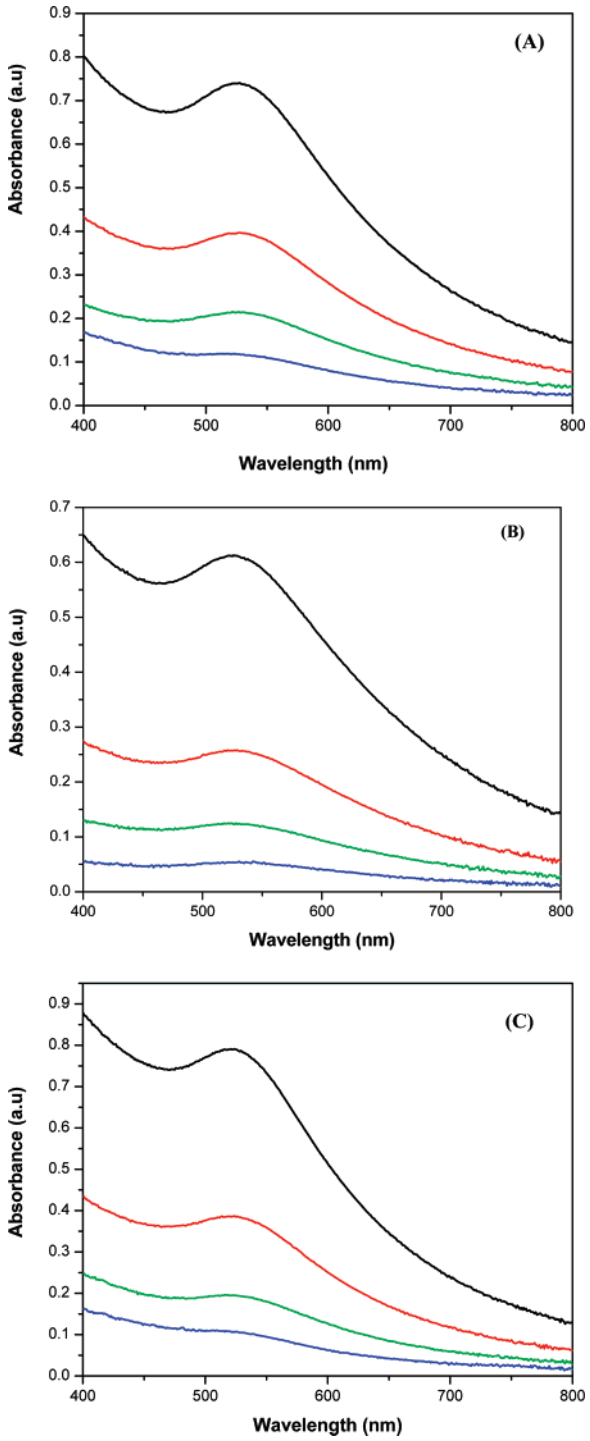

Figure 3. UV−vis spectra of functionalized gold nanoparticles solution as a function of concentration for the following samples: **GNP-5**, molar ratio of B-poly(NAM-*co*-GalAm)-1/PNIPAAm-1/MPEG-SH = 1:2:2 (A); **GNP-6**, molar ratio of B-poly(NAM-*co*-GalAm)-2/PNIPAAm-4/MPEG-SH (M_n = 20 000 g.mol^{-1}) = 0.5:1:1 (B); **GNP-7**, molar ratio of poly(NAM-*co*-GalAm)/PNIPAAm-1/MPEG-SH = 0.5:1:1 (C).

PNIPAAm (110 μM), MPEG-SH (110 μM), and the glycopolymer (55 μM) were mixed together in an aqueous solution containing $HAuCl_4$ (330 μM) and Irgacure-2959 (1 mM). This mixture was stirred at room temperature for 10 min. The clear solution was then irradiated for a few minutes in a Rayonet photoreactor (Southern N.E. Ultraviolet Co.) using 16 75W UV lamps having a 350 nm wavelength. The color of the final solution changes to purple or pink

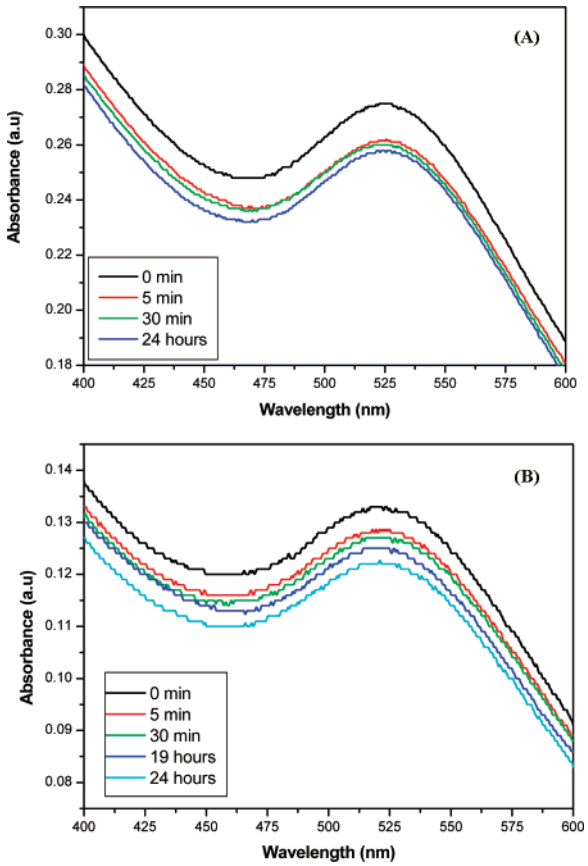

Figure 4. Change in absorbance with time on addition of 100 μg.mL^{-1} of streptavidin to the gold glyconanoparticles solution: sample **GNP-5** (A); sample **GNP-6** (B).

indicating the formation of gold nanoparticles. Moreover, the gold nanoparticle solution showed very good colloidal stability over several weeks.

Results and Discussion

Biotinylated glycopolymers and PNIPAAm were synthesized by RAFT polymerization. RAFT is a versatile control radical polymerization process that can be applied to many different monomers including polar ones. Moreover, it is rather easy to introduce a ligand at the α-chain-end using an appropriate functional RAFT agent. Here, we use a biotin chain transfer agent (biotin-CTA) previously synthesized from a precursor RAFT agent[67] (see Figure 1), that possesses stable bonds between the biotin moiety and the dithioester moiety (amide and ether bonds) (Scheme 1). This biotin-CTA proved to be very efficient for the synthesis of homopolymers of *N*-acryloylmorpholine (NAM)[67] and for the copolymerization of NAM with a protected vinyl galactose derivative.[68] RAFT copolymerization reached 80% conversion, and well-controlled polymer chains were obtained (Table 1 and Figure 2). There are several reports in the literature detailing the RAFT polymerization of NIPAAM.[46−60] In this work, we have focused on the synthesis NIPAAM homopolymers via the RAFT process using *S*,*S*′-bis(α,α′-dimethyl-α″-acetic acid)trithiocarbonate (CTA_1) as the chain transfer agent. 4,4-Azobis(4-cyanovaleric acid) (ACVA) was used as the azo initiator, and the polymerizations were performed at 65 °C in dioxane. Other details of the synthesis are shown in Scheme 2 and Table 2.

After deprotection of the sugar rings as shown in Scheme 1, two samples of biotinylated glycopolymer, B-poly(NAM-*co*-GalAm)-1 and -2 (Table 1), together with the RAFT-prepared PNIPAAm and methoxy-capped PEG (MPEG-SH, M_n = 5000 or 20 000 g.mol^{-1}) were chosen for the preparation of gold glyconanoparticles. The short chains (M_n = 2000 g.mol^{-1}) and longer chains (M_n = 43 900 g.mol^{-1}) of the biotinylated glycopolymers correspond to 2 and 50 sugar residues per chain, respectively. In addition, a control glycopolymer sample was also chosen with a *tert*-butyl α-end-group instead of a biotin, for the preparation of the glyconanoparticles. The non-biotinylated glycopolymer (M_n = 22 600 g.mol^{-1}) has an average of 20 sugar residues per chain. This sample has been used during the SPR experiments to evaluate the possible nonspecific interactions between the glyconanoparticles and the streptavidin-covered surface of the SPR sensor chip.

The well-established thiol chemical method for gold stabilization is applied here for the formation of gold glyconanoparticles. Glycopolymers, PNIPAAm, and MPEG-SH have all been individually used in the stabilization of gold nanoparticles.[18,23,61−65] Here, we demonstrate for the first time the formation of stable gold colloids with a mixture of the glycopolymers, PNIPAAm, and MPEG-SH via a photochemical process. McGilvray and co-workers[71] have recently shown the formation of stable gold nanoparticles via a photochemical reduction of $HAuCl_4$ with Irgacure-2959. Irgacure-2959 is a water-soluble photoinitiator and decomposes quantitatively in the presence of UV light at a wavelength of 350 nm. Highly monodisperse gold colloids were obtained by this photochemical method. Here, we used the photochemical initiator, Irgacure-2959, for the in situ reduction of the RAFT-prepared polymers, MPEG-SH and $HAuCl_4$, for the preparation of functionalized monodisperse gold colloids. A mixture of Irgacure-2959, $HAuCl_4$, glycopolymer, PNIPAAm, and MPEG-SH was first dissolved in filtered distilled deionized water, and the resulting pale yellow solution was then placed in a photochemical reactor for a period of approximately 5−10 min. The clear solution changes from pale yellow to pink indicating the formation of gold nanoparticles. For purification, the gold colloid solution was then dialyzed against water. It should be noted that the in situ reduction minimizes the formation of disulfide bridges between the thiol-terminated polymers. The

Table 3. Synthetic Parameters for the Formation of the Glyconanoparticles

sample	M_n PNIPAAm (g.mol^{-1})	M_n of MPEG-SH (g.mol^{-1})	M_n of glycopolymer (g.mol^{-1})	molar ratio glycopolymer/ PNIPAAm/MPEG-SH	DLS (nm)	PDI
GNP-1	19 169				158	0.26
GNP-2	15 000				117	0.33
GNP-3	6540				159	0.37
GNP-4	6201				125	0.20
GNP-5	6540	5000	2000 B-poly(NAM-*co*-GalAm)-1	1:2:2	97	0.37
GNP-6	19 169	20 000	43 900 B-poly(NAM-*co*-GalAm)-2	0.5:1:1	83	0.30
GNP-7	19 169	20 000	22 600 poly(NAM-*co*-GalAm)	0.5:1:1	128	0.11

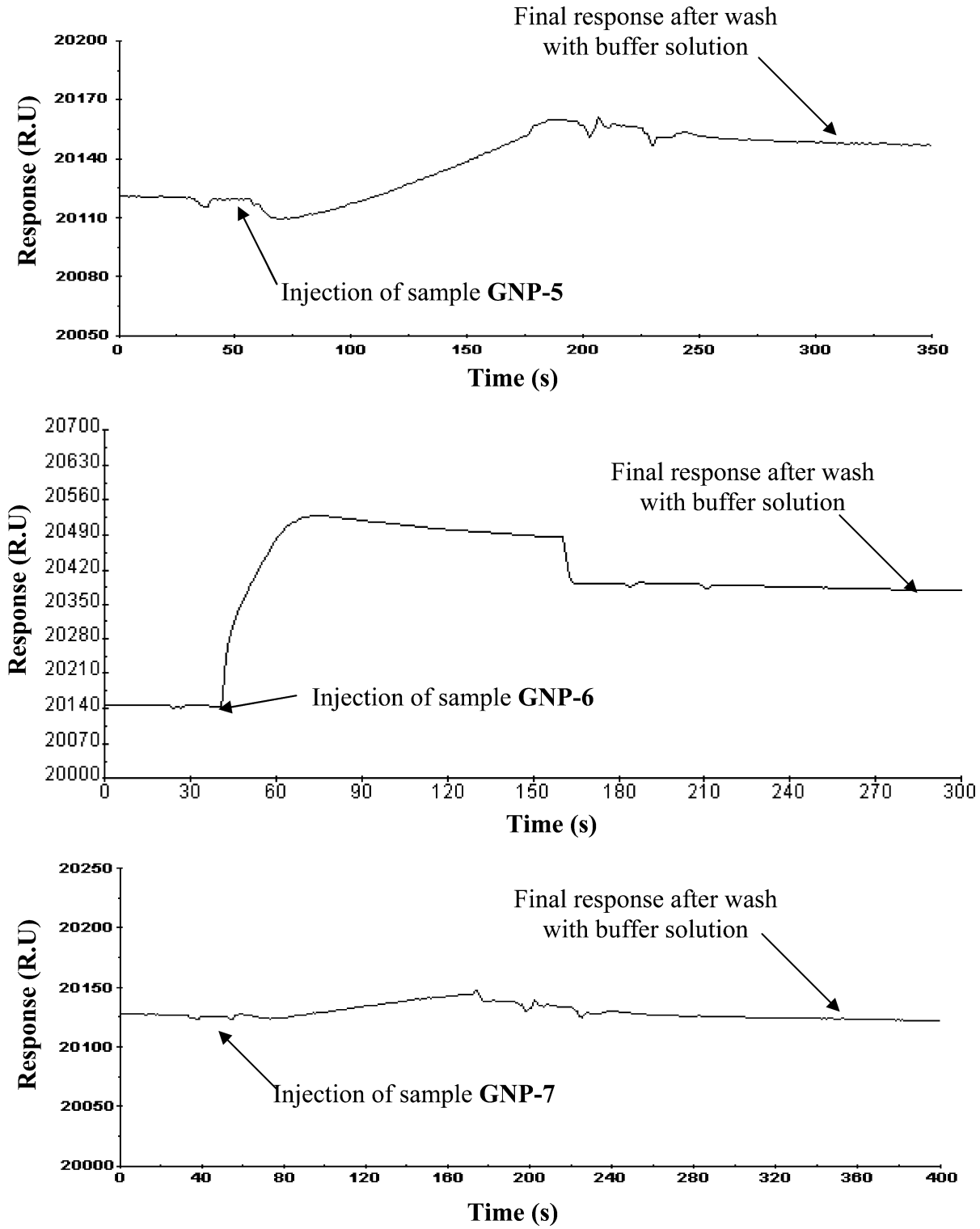

Figure 5. SPR sensorgram for the bioconjugation of the biotinylated (**GNP-5** and **GNP-6**) and non-biotinylated gold glyconanoparticles (**GNP-7**) on streptavidin immobilized sensor chip.

gold colloids were analyzed by dynamic light scattering (DLS) and UV−vis spectroscopy (see Figure 4 and Table 3). The aqueous solution of the gold nanoparticles showed excellent stability over weeks, as no aggregation was noted by dynamic light scattering. It should be noted that when only PNIPAAm homopolymers were used for the stabilization of the gold nanoparticles, the nanoparticles were larger and polydisperse as revealed by dynamic light scattering (DLS) measurements. However, a mixture of the glycopolymer, PNIPAAm, and MPEG-SH resulted in the formation of relatively smaller-sized gold nanoparticles.

Finally, we investigated the accessibility of the biotin ligands on the surface of the gold nanoparticles for interaction with streptavidin. Streptavidin has been used widely as a model protein in a number of biorelated applications such as affinity separations, bioassays, and clinical diagnostics because of its high affinity to biotin (association constant = 10^{13-15} M^{-1}). We have used

(71) McGilvray, K. L.; Decan, M. R.; Wang, D.; Scaiano, J. C. *J. Am. Chem. Soc.* **2006**, *128*, 15980−15981.

spectrophotometry and surface plasmon resonance (SPR) to study the interactions of biotin on the nanoparticles to streptavidin. We have also used two types of biotinylated gold glyconanoparticles (**GNP-5** and **GNP-6**) where the biotin is connected to either a short or long polymer chain. Furthermore, we have also used non-biotinylated gold nanoparticles (**GNP-7**) as a control for any nonspecific interactions of the nanoparticles with streptavidin. The addition of streptavidin to a solution of biotinylated gold nanoparticles dispersion (samples **GNP-5** or **GNP-6**) was monitored spectrophotometrically over time to record any changes in the plasmon absorption (see Figure 4). A shift of the maximum absorbance to lower values was noted with time, which is an indication of the aggregation of the nanoparticles induced by the addition of the streptavidin. This shows that the biotin is accessible on gold nanoparticle samples **GNP-5** and **GNP-6**. Addition of streptavidin to non-biotinylated gold dispersions showed no change in absorbance over time, which indicates that the aggregation is only caused by the conjugation of the streptavidin to biotin ligands present on the surface of the nanoparticles. It is interesting to note that the bathochromic shift is only very small probably due to the larger size of the nanoparticles (∼90−100 nm) as compared to streptavidin (3−4 nm). These results are also consistent to our previously reported results for the interaction of biotinylated glyconanoparticles with streptavidin.[23] Ishii and co-workers[72] previously reported a larger bathochromic shift over time for the aggregation of biotinylated PEGylated gold nanoparticles (∼10 nm) with streptavidin. These results are further supported by a surface plasmon resonance (SPR) study involving the biotinylated and non-biotinylated gold dispersions. SPR is a robust technique to study biomolecular events in real time. In particular, SPR has extensively been used to study the biomolecular interactions between biotinylated macromolecules or nanoparticles to streptavidin. We investigated the accessibility of the biotin ligands on the glyconanoparticles to bind to a streptavidin coated sensor chip. The biotinylated (**GNP-5** and **GNP-6**) and non-biotinylated (**GNP-7**) glyconanoparticles were injected separately into the SPR instrument containing a preloaded streptavidin-coated sensor chip. After injection of the gold dispersions, an immediate response was observed, and after automatic wash (with buffer), the difference in response is indicative of the binding of the biotinylated glyconanoparticles on the sensor chip. For biotinylated samples **GNP-5** and **GNP-6**, a difference in response was observed even after automatic wash with buffer solution as shown in Figure 5. This difference in response is indicative of the bioconjugation of the biotinylated nanoparticles on the streptavidin coated sensor chip. This shows that the biotin is accessible to bind to surface-immobilized streptavidin. As a control for the non-biotinylated gold nanoparticle sample **GNP-7**, no change in response was observed after buffer wash, and this result is consistent with the UV−vis data. Furthermore, the gold nanoparticles do not show any nonspecific binding to the sensor chip surface.

(72) Ishii, T.; Otsuka, H.; Kataoka, K.; Nagasaki, Y. *Langmuir* **2004**, *20*, 561−564.

Conclusions

We have successfully prepared biotinylated glycopolymers and PNIPAAm via the reversible addition fragmentation chain transfer polymerization process. We have also demonstrated for the first time that the RAFT prepared polymers can be photochemically reduced in situ for the preparation of gold nanoparticles. Photochemically prepared gold nanoparticles with a surface composition of PNIPAAm, glycopolymer, and MPEG-SH revealed high colloidal stability over a long period of time. Furthermore, we have also shown that the biotin ligands on the surface of the nanoparticles are still accessible for bioconjugation to streptavidin.

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