Synthesis of Highly Water-Soluble Fluorescent Conjugated Glycopoly(*p*-phenylene)s for Lectin and *Escherichia coli*

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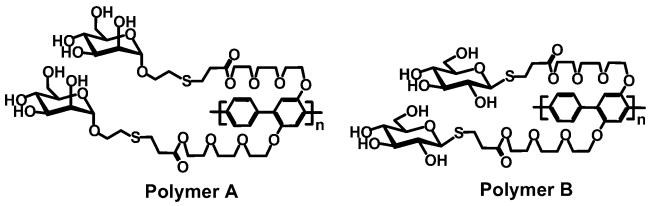
Two facile, convenient, and versatile synthetic approaches are used to covalently attach carbohydrate residues to conjugated poly(*p*-phenylene)s (PPPs) for highly water-soluble PPPs bearing α-mannopyranosyl and *β*-glucopyranosyl pendants (polymers **A** and **B**), which highly fluoresce in phosphate buffer (pH 7.0). The postpolymerization functionalization approach is to treat bromo-bearing PPP (polymer **1**) with 1-thiolethyl-α-D-mannose tetraacetate or 1-thiol-*β*-D-glucose tetraacetate in THF solution in the presence of K_2CO_3 at room temperature through formation of thioether bridges, affording polymer **2a** or **2b**. The prepolymerization functionalization approach is to polymerize a well-defined sugar-carrying monomer, affording polymer **2a**. Polymers **2a** and **2b** were deacetylated under Zemplén conditions in methanol and methylene chloride containing sodium methoxide, affording polymers **A** and **B**, respectively. The multivalent display of carbohydrates on the fluorescent conjugated glycopolymer overcomes the characteristic low binding affinity of the individual carbohydrates to their receptor proteins. Titration of concanavalin A (Con A) to α-mannose-bearing polymer **A** resulted in significant fluorescent quenching of the polymer with Stern−Volmer quenching constant of 4.5×10^7. Incubation of polymer **A** with *Escherichia coli (E. coli)* lead to formation of fluorescently stained bacterial clusters. *β*-Glucose-bearing polymer **B** displayed no response to Con A and *E. coli*.

Introduction

Protein−carbohydrate interactions are involved in a wide variety of cellular recognition processes including cell growth regulation, differentiation, adhesion, cancer cell metastasis, cellular trafficking, inflammation by bacteria and viruses, and the immune response.[1,2] These specific interactions occur through glycoproteins, glycolipids, and polysaccharide displays found on cell surfaces and proteins with carbohydrate-binding domains called lectins.[3] However, since it is known that individual carbohydrate-protein interactions are generally weak, it is important to investigate how such weak carbohydrate−protein interactions can be amplified by the glycoside clusters expressed on the cell surface, and how the overall binding capacity with protein receptors, commonly with multiple binding sites, is enhanced over the affinity of individual monovalent ligands through cooperative multiple interactions.[3] Multivalent forms of carbohydrate ligands, either glycopolymers or glycodendrimers, have been employed to demonstrate that inhibitory potencies of glycosides are enhanced over their monovalent counterparts although the levels of enhancement vary.[4−6] Fluorescent conjugated glycopolymers combining scaffolding and reporting functions into one package are very attractive for biosensing applications because of their intrinsic fluorescence and their high sensitivity to minor external stimuli due to amplification by a cooperative system response.[7−9] Mannose-grafted poly(*p*-phenylene-ethynylene)s (PPEs) have been prepared to successfully detect Con A and *E. coli* by polymerization of well-defined sugar-carrying monomers[9] or partially chemical modification of PPE bearing carboxylic acid groups with amine-functionalized carbohydrates through amide bridges.[8] Neutral PPEs bearing carbohydrate pendants display low water solubility,[10] probably due to strong $\pi-\pi$ stacking interactions of hydrophobic PPE backbones. Introduction of anionic groups such as carboxylic acid to PPE significantly enhances water solubility of carbohydrate-bearing PPEs by preventing the $\pi-\pi$ stacking interactions of PPE backbones via charge repulsion.[11] However, the presence of ionic groups in conjugated polymers might cause interfering responses due to nonspecific electrostatic interactions in complicated biological samples.[11]

Scheme 1. Chemical Structures of α-Mannose-Bearing Polymer A and *β*-Glucose-Bearing Polymer B



In this communication, we choose poly(*p*-phenylene)s (PPPs) to enhance water-solubility of neutral conjugated glycopolymers because they display relatively weaker $\pi-\pi$ stacking interactions among polymer backbones than PPEs due to the rotatable phenylene groups in the PPP backbone.[12] We present simple, convenient, and versatile prepolymerization and postpolymerization functionalization methods to develop water-soluble neutral fluorescent α-mannose-bearing PPP (polymer **A**) and *β*-glucose-bearing PPP (polymer **B**) to detect Con A and *E. coli* by multivalent cooperative interactions *(*Scheme 1*).* Polymers **A** and **B** not only display high solubility in water but also highly fluoresce in phosphate buffer solution (pH 7.2). The presence of Con A significantly quenches the fluorescence of α-mannose-bearing polymer **A** with a Stern−Volmer quenching constant of 4.5×10^7, whereas titration of Con A to a phosphate buffer solution containing *β*-glucose-bearing polymer **B** shows no change in the polymer fluorescence. Polymer **A** binds specifically to FimH adhesion of bacterial type 1 pili, resulting in fluorescently stained bacteria clusters and shows significantly stronger interactions with FimH protein compared with free

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mannose in the competition assay. However, polymer **A** displays no response to mutant ORN208 strain which is deficient in the fimH gene and expresses abnormal type 1 pili that fail to mediate D-mannose-specific binding.

Experimental Section

Instrumentation. ^{1}H NMR and ^{13}C NMR spectra were taken on a 400 MHz Varian Unity Inova spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, and chemical shifts (δ) are given in ppm relative to solvent peaks (^{1}H, δ 7.26; ^{13}C:, δ 77.3) as internal standard. UV spectra were taken on a Hewlett-Packard 8452A Diode Array UV−visible spectrophotometer. A total of 10 mL of stock solution was prepared with 10 mg/mL of Con A in phosphate buffer solution (PBS; pH of 7.2) and used to titrate into a PBS solution of α-mannose-functionalized polymer (1.0×10^{-7} mol/L) in the presence of 0.1 mM $CaCl_2$ and 0.1 mM $MnCl_2$. Fluorescence spectra were obtained on a Spex Fluorolog 1681 0.22 m steady-state fluorometer. Molecular weights of the polymers were determined by gel permeation chromatography (GPC) by using a Waters Associates model 6000A liquid chromatograph. Three American Polymer Standards Corp. Ultrastyragel columns in series with porosity indices of 10^3, 10^4, and 10^5 Å were used and housed in an oven thermostated at 30 °C. The mobile phase was HPLC grade THF which was filtered and degassed by vacuum filtration through a 0.5 μm Fluoropore filter prior to use. The polymers were detected by a Waters model 440 ultraviolet absorbance detector at a wavelength of 254 nm and a Waters model 2410 refractive index detector. Molecular weight was measured relative to polystyrene standards.

Cell growth. Two *E. coli* bacterial strains that differ only in their mannose-binding ability, ORN178, a mannose-binding strain and ORN208, a mutant strain that does not bind mannose, were used for this study. ORN178 and ORN208 are generous gifts from Professor Paul Orndorff, North Carolina State University. Cells were grown in sterile Luria-Bertani (LB) media (containing bacto-tryptone (2.5 g), bacto-yeast extract (1.25 g), and NaCl (2.5 g) in 250 mL of deionized water adjusted to pH to 7.0 with 5 M NaOH). A single colony of each strain was lifted from agar plates and inoculated in 10 mL of LB media and the culture was grown overnight until the A_{600} reached 1.0. Aliquots of cells (1 mL) were centrifuged at 10 000g for 30 min at 4 °C and washed twice with 1 mL of PBS buffer (containing 100 mM Na_2HPO_4, 1.75 mM KH_2PO_4, 140 mM NaCl, and 2.70 mM KCl adjusted to pH 7.2 using HCl). Washed cells were resuspended in 1-mL PBS buffer containing $CaCl_2$ (1 mM) and $MnCl_2$ (1 mM). Cells were incubated with 10−20 μg of polymer for 30 min with gentle shaking, centrifuged at 10 000*g* for 30 min, and washed four times with PBS buffer and the final cell pellet was resuspended in PBS buffer.

Materials. Unless otherwise indicated, all reagents, solvents, and proteins were obtained from commercial suppliers (Aldrich, Sigma, Fluka, Acros, and Lancaster) and were used without further purification. Air- and moisture-sensitive reactions were conducted in oven-dried glassware using standard Schlenk line or drybox techniques under a nitrogen atmosphere. Monomer **1**, 1-thiolethyl-α-D-mannose tetraacetate (**1a**) and 1-thiolethyl-α-D-mannose were prepared and characterized according to literatures.[13−15]

Monomer 2. Monomer **1** (0.5 g, 0.56 mmol), 1-thiolethyl-α-D-mannose tetraacetate (0.5 g, 1.16 mmol), and potassium carbonate (0.8 g, 5.80 mmol) were placed in a 100 mL round-bottom flask. THF (50 mL) was added to the flask, and the reaction vessel was degassed and refilled with N_2. After the mixture was stirred at room temperature for 24 h, the solvent was removed and the residue was diluted with methylene chloride (50 mL), washed with water, and dried over anhydrous $MgSO_4$. The solvent was evaporated, and the crude compound was purified by column chromatography on silica gel with CH_2Cl_2/EtOAc (5:3) to give the target compound (0.81 g, 91% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.22 (s, 2H), 5.26−5.29 (m, 4H), 5.22 (m, 2H), 4.83 (s, 2H), 4.24−4.26 (m, 6H), 4.07−4.10 (m, 8H), 3.85−3.87 (m, 4H), 3.79−3.82 (m, 2H), 3.75−3.77 (m, 4H), 3.70−3.72 (m, 4H), 3.65−3.68 (m, 6H), 2.80−2.82 (t, J = 7.2 Hz, 4H), 2.73−2.76 (t, J = 6.8 Hz, 4H), 2.62−2.65 (t, J = 6.8 Hz, 4H), 2.13 (s, 6H), 2.08 (s, 6H), 2.03 (s, 6H), 1.97 (s, 6H) ppm. ^{13}C NMR (400 MHz, $CDCl_3$): δ 171.91, 170.83, 170.21, 170.03, 169.94, 153.30, 123.66, 97.84, 86.61, 71.35, 70.90, 70.49, 69.83, 69.70, 69.35, 69.21, 68.94, 68.13, 66.27, 64.08, 62.67, 35.01, 31.55, 27.65, 21.08, 20.96, 20.91, 20.87 ppm. HRMS: Calcd, 1551.2343 for $[M + H]^+$ ($C_{56}H_{81}I_2O_{30}S_2$). Found, 1551.2347.

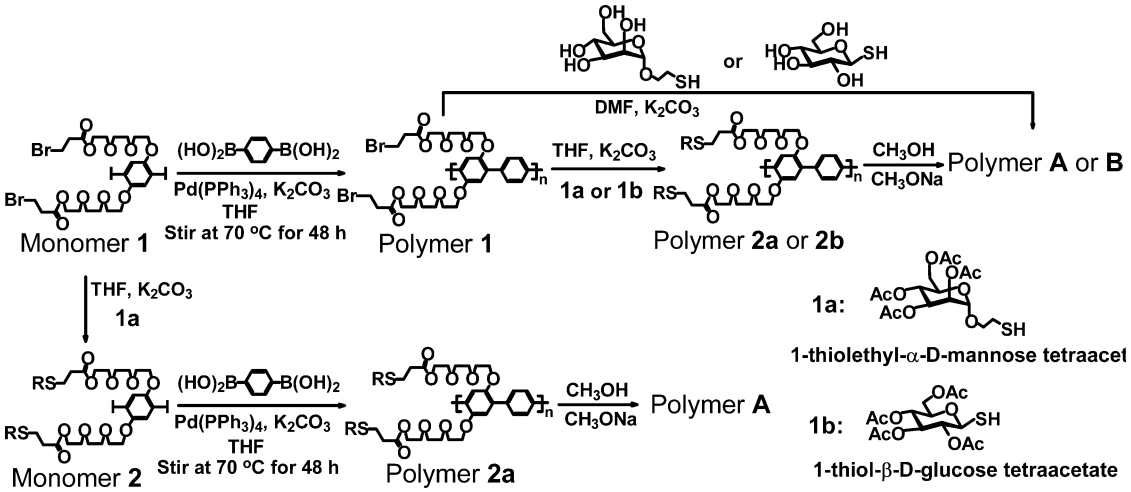
Polymer 1. Under a nitrogen atmosphere, monomer **1** (1.20 g, 1.34 mmol), 1,4-phenyldiboronic acid (0.28 g, 1.68 mmol), $Pd(PPh_3)_4$ (10 mg), and potassium carbonate (2.50 g, 18.12 mmol) were placed in a 50 mL round-bottom flask, and THF (45 mL) was added. The mixture was stirred at 70 °C for 48 h under nitrogen and then precipitated by addition to methanol. The polymer was filtered, washed with methanol and acetone, and then dried under vacuum for 24 h to afford the neutral precursor (polymer **1**) (700 mg, 73%) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53 (broad, 4H), 7.29 (broad, 2H), 4.26 (m, 4H), 4.11 (m, 4H), 3.47−3.82 (m, 20H), 2.89 (m, 4H) ppm. GPC (THF, polystyrene standard), M_n: 28 500 g/mol; PDI: 1.75. Polymer **1** displays absorption maxima at 339 nm and emission maxima at 440 nm in chloroform solution.

Polymer A. Polymer **A** was prepared by postpolymerization functionalization of the polymer **1** via thioether formation of the bromoalkyl groups with thiol-functionalized carbohydrate. Polymer **1** (210 mg), 1-thiolethyl-α-D-mannose tetraacetate (600 mg, 1.38 mmol), and potassium carbonate (0.35 g, 3.27 mmol) were placed in a 50 mL round-bottom flask. THF (35 mL) was added to the flask and the reaction vessel was degassed and refilled with N_2. The mixture was stirred at room temperature for 48 h. When the solvent was removed, the residue was diluted with methylene chloride (50 mL), washed with water, and dried over anhydrous $MgSO_4$. The filtrate was concentrated and the residue was purified by flash column chromatography with EtOAc to remove the excess 1-thiolethyl-α-D-mannose tetraacetate and then eluted with CH_2Cl_2/CH_3OH (1:1) to give the polymer **2a** (0.30 g, 72.5% yield) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (broad, 4H), 7.30 (broad, 2H), 5.30−5.22 (m, 6H), 4.83 (s, 2H), 4.24−4.10 (m, 12H), 4.05−3.65 (m, 20H), 2.80−2.62 (m, 16H), 2.13−1.97 (m, 24H) ppm. Polymer **2a** (200 mg) was dissolved in mixture of CH_2Cl_2 (20 mL) and CH_3OH (40 mL), and CH_3ONa in CH_3OH solution (6 mL) was added. The resulting mixture was stirred at room temperature under N_2 for 24 h. After the organic solvent was evaporated, the resulting residue was dissolved in 10 mL of water, dialyzed in a cellulose dialysis tube (cutoff 12 000) against water for 2 days (10 water changes), and lyophilized to give polymer **A** (0.14 g, 91.1%) as a yellow solid. ^{1}H NMR (400 MHz, D_2O): δ 7.60, 7.14, 4.0−4.11, 3.43−3.77, 2.46 ppm. Polymer **A** is highly soluble in water and displays absorption maxima at 338 nm and emission maxima at 438 nm in phosphate buffer (pH 7.0).

Polymer **A** was also prepared by postpolymerization functionalization of the polymer **1** via thioether formation of the bromoalkyl groups with 1-thiolethyl-α-D-mannose. Polymer **1** (210 mg), 1-thiolethyl-α-D-mannose (700 mg), and potassium carbonate (0.75 g) were placed in a 50 mL round-bottom flask. DMF (35 mL) was added to the flask, and the reaction flask was degassed and refilled with N_2. After the mixture was stirred at room temperature for 72 h, the resulting mixture was dialyzed in a cellulose dialysis tube (cutoff 12 000) against water for 3 days (15 water changes), and lyophilized to give polymer **A**. Its NMR data are the same as those above.

Polymer **A** was also prepared through prepolymerization functionalization by polymerizing well-defined sugar-carrying monomer **2**. Under a nitrogen atmosphere, monomer **2** (0.5 g, 0.31 mmol), 1,4-phenyldiboronic acid (0.06 g, 0.36 mmol), $Pd(PPh_3)_4$ (80 mg), and potassium carbonate (0.5 g, 3.62 mmol) were placed in a 50 mL round-bottom flask, and THF (20 mL) was added. The mixture was stirred at 70 °C for 48 h under nitrogen atmosphere. The purification of polymer **2a** was conducted according to the procedure above. ^{1}H NMR data of

Scheme 2. Synthetic Route to Fluorescent Conjugated Glycopoly(*p*-phenylene)s



polymer **2a** are the same as that obtained by post-polymerization functionalization of polymer **1** with 1-thiolethyl-α-D-mannose tetraacetate (**1a**). Polymer **A** was obtained by de-acetylation under Zemplén conditions in methanol and methylene chloride containing sodium methoxide and its NMR data are the same as those obtained by the postpolymerization functionalization above.

Polymer B. Polymer **B** was prepared in a similar way to polymer **A** except using 1-thiol-β-D-glucose tetraacetate (**1b**) instead of 1-thiolethyl-D-mannose tetraacetate (**1a**). ^{1}H NMR (400 MHz, D_2O): δ 7.60, 7.27, 4.09−3.95, 3.80−3.40, 2.42 ppm. Polymers **B** is highly soluble in water and displays absorption maxima at 339 nm and emission maxima at 439 nm in phosphate buffer (pH 7.2).

Polymer **B** was also prepared by postpolymerization functionalization of the polymer **1** via thioether formation of the bromoalkyl groups with 1-thiol-β-D-glucose. Polymer **1** (200 mg), 1-thiol-β-D-glucose sodium salt hydrate (650 mg), and potassium carbonate (0.75 g) were placed in a 50 mL round-bottom flask. DMF (35 mL) was added to the flask, and the reaction flask was degassed and refilled with N_2. After the mixture was stirred at room temperature for 72 h, the resulting mixture was dialyzed in a cellulose dialysis tube (cutoff 12 000) against water for 3 days (15 water changes) and lyophilized to give Polymer **B**. Its NMR data are the same as those above.

Results and Discussion

We explored simple, convenient, versatile prepolymerization and postpolymerization functionalization methods to attach carbohydrate residues to a poly(*p*-phenylene) (PPP) scaffold through flexible oligo(ethylene glycol) tethers. The prepolymerization functionalization method is to prepare a well-defined α-mannoside-carrying diiodoaryl monomer (**2**) and polymerize it with 1,4-phenyldiboronic acid by the palladium-catalyzed Suzuki coupling reaction, affording PPP bearing peracetylated α-mannoside residues (polymer **2a**), which was deacetylated under Zemplén conditions in a mixed solution of methanol and methylene chloride containing sodium methoxide, resulting in α-mannose-bearing polymer **A** (Scheme 2). Monomer **2** was readily obtained through reaction of bromide-bearing monomer (**1**) with 1-thiolethyl-α-D-mannose tetraacetate in THF solution in the presence of K_2CO_3, forming thioether bridges (Scheme 2). This approach using well-defined and anomerically pure building blocks can create well-defined molecular constitution of fluorescent conjugated glycopolymers. The postpolymerization functionalization approach is to treat bromide-bearing polymer **1** with 1-thiolethyl-α-D-mannose tetraacetate (**1a**) or 1-thiol-β-D-glucose tetraacetate (**1b**) in the THF solution in the presence of K_2CO_3, resulting in PPPs bearing peracetylated α-mannoside or β-glucoside residues (polymer **2a** or **2b**), which were deacetylated under Zemplén conditions in a mixed solution of methanol and methylene chloride containing sodium methoxide, resulting in α-mannose-bearing PPP (polymer **A**) or β-glucose-bearing PPP (polymer **B**) (Scheme 2). The bromide-bearing polymer **1** was obtained by polymerization of a bromide-bearing diiodoaryl monomer (**1**) and 1,4-phenyldiboronic acid in the palladium-catalyzed Suzuki coupling reaction condition (Scheme 2). The latter methodology is generally advantageous because it needs fewer reaction steps than the prepolymerization functionalization method, allows easy control of the number of sugars along the polymeric chain and provides an effective means to rapidly attach a variety of different carbohydrates to conjugated polymers. Polymer **1** displays absorption maxima at 339 nm and emission maxima at 440 nm in chloroform solution, which were ascribed to the $\pi-\pi^*$ transition of the conjugated polymer backbone. Polymer **A** exhibits absorption maxima at 338 nm and emission maxima at 438 nm in phosphate buffer solution (pH 7.2). Polymer **B** displays absorption maxima at 339 nm and emission maxima at 439 nm in phosphate buffer (pH 7.2). Polymers **A** and **B** were found to be very stable in deionized water and phosphate buffer solution (PBS). Moreover, they are easily dissolved in aqueous media without aggregation and highly fluorescent in phosphate buffer (pH 7.2). These properties have made their applications in biological systems feasible.

We used the fluorescent α-mannose-bearing polymer **A** to study its ability to recognize Con A and β-glucose-bearing polymer **B** as a control polymer to test selectivity. Con A, a member of the lectin family, selectively binds to α-mannopyranosyl and α-glucopyranosyl residues.[16] It exists predominantly as a tetramer of four identical subunits of approximately 26 000 Da at neutral and alkaline pH levels.[16] Below pH 5.6, however, it dissociates into active dimers. It binds two metal ions per monomer; a transition metal, Mn^{2+} and Ca^{2+}, must be present for saccharide binding.[16] Titration of Con A into a phosphate buffer solution containing α-mannose-bearing polymer **A** results in a concentration-dependent quenching of the polymer fluorescence (Figure 1) and a very small decrease of the polymer UV−visible absorption. However, titration of Con A into a phosphate buffer solution containing β-glucoside-bearing polymer **B** shows no significant change of the polymer fluorescence. This is consistent with the literature which showed that Con A displays no binding ability to β-glucose.[17] These

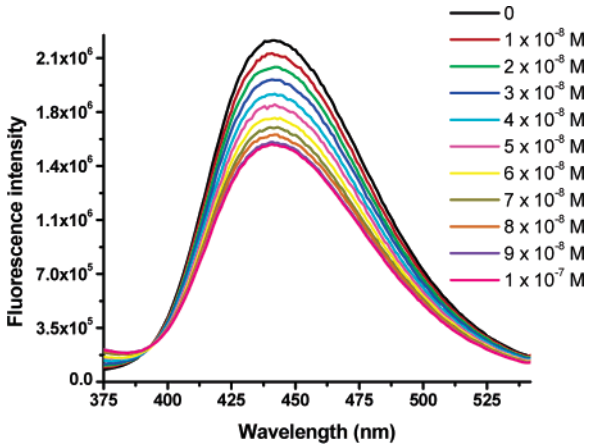


Figure 1. Fluorescent spectra of polymer **A** in the absence and presence of Con A in a phosphate buffer solution containing 1.0×10^{-7} M polymer **A**, 0.1 mM $CaCl_2$, and 0.1 mM $MnCl_2$.

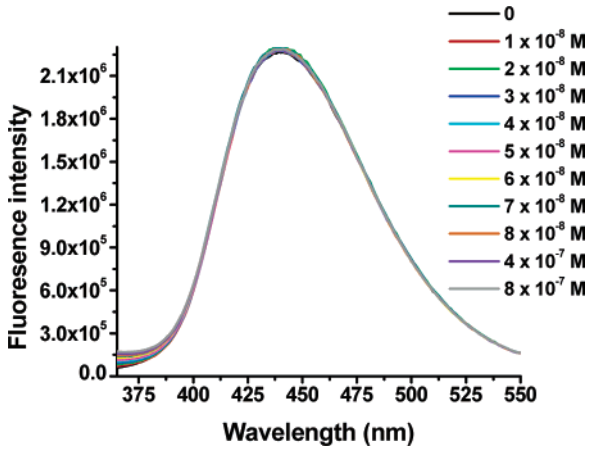


Figure 2. Fluorescent spectra of polymer **B** in the absence and presence of Con A in a phosphate buffer solution (pH 7.2) containing 1.0×10^{-7} M polymer **B**, 0.1 mM $CaCl_2$, and 0.1 mM $MnCl_2$.

results clearly indicate that the α-mannose-bearing polymer **A** selectively binds Con A, resulting in quenching of the polymer fluorescence and that the polymer does not possess any nonspecific binding to Con A.

Conjugated polymers feature short emissive lifetimes on the order of 0.2−0.5 ns unless they contain organometallic fragments.[12,18,19] As a result, only static quenching is predominant.[12,18,19] In static quenching, the quencher forms a ground-state complex with the fluorophore which is then quenched after excitation: quenching constant K_{SV} in static quenching equals the apparent complex formation constant of quencher to fluorophore. Using the Stern−Volmer relationship offers a simple way to extract binding constants.[20] A quantitative measure of the fluorescence quenching can be achieved by determining the well-known Stern−Volmer constant, K_{SV}[20]

$$I_0/I = 1 + K_{SV}[Q]$$

where I_0 is the intensity of fluorescence in the absence of the quencher and I is the intensity of fluorescence in the presence of the quencher. [Q] is the concentration of quencher. The equation reveals that I_0/I increases in direct proportion to the concentration of the quencher, and K_{SV} is the Stern−Volmer constant, defining the efficiency of quenching. When all other variables are held constant, the higher the K_{SV}, the lower the concentration of quencher required to quench the fluorescence.[20] The Stern−Volmer quenching constant of polymer **A** by Con A was calculated as 4.5×10^7 (Figure 3).

Type 1 pili in *Escherichia coli* (*E. coli*) are filamentous proteinaceous appendages composed of FimA, FimF, FimG, and FimH proteins. FimA accounts for more than 98% of the pilus protein, and FimH is uniquely responsible for the binding to D-mannose. We choose two *E. coli* strains ORN178 and ORN208 for testing and control experiments to investigate the specific binding of the α-mannose-bearing polymer **A** to FimH protein. The ORN178 strain expresses the wild-type type 1 pili that selectively bind mannose, whereas the ORN208 strain is deficient of the fimH gene and expresses abnormal type 1 pili that fail to mediate D-mannose-specific binding. These bacteria strains in 1 mL of PBS buffer were individually incubated with 10−20 μg of polymer **A** or **B** for 30 min with gentle shaking, centrifuged at 10 000*g* for 30 min, washed five times with PBS buffer and the final cell pellet was resuspended in PBS buffer. Incubation of polymer **A** with ORN178 strain resulted in formation of fluorescently stained bacteria clusters from which

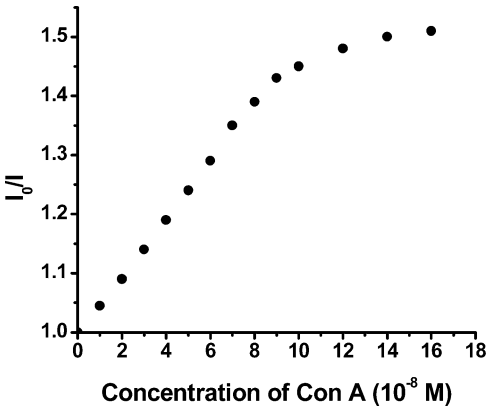


Figure 3. Stern−Volmer curve of polymer A with different Con A concentrations in phosphate buffer containing 1.0×10^{-7} mol/L of polymer **A**, 0.1 mM $CaCl_2$, and 0.1 mM $MnCl_2$.

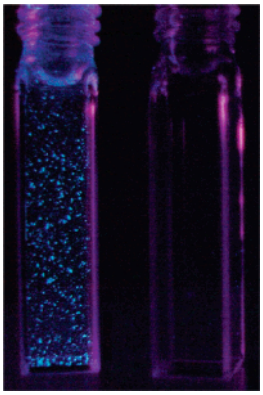


Figure 4. Visualization of mannose-binding ORN178 strain (left) and mutant ORN208 strain (right) after incubation with α-mannose-bearing polymer **A**.

the polymer was not removed by rinsing and separation (Figure 4). However, polymer **B** did not bind the ORN178 strain.

The presence of the ORN178 strain in polymer **A** in phosphate buffer solution (pH 7.0) results in a significant quenching of the polymer fluorescence (Figure 5), whereas the presence of the ORN208 strain shows no significant change in the polymer fluorescence. In addition, the presence of the ORN178 strain in phosphate buffer solution (pH 7.2) containing β-glucose-bearing polymer **B** displays no significant change in the polymer fluorescence. The experimental results indicate that polymer **A** selectively binds the pili of the ORN178 strain but

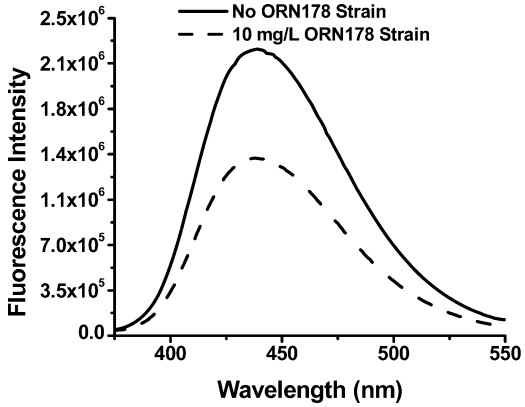


Figure 5. Fluorescence spectra of polymer **A** in the absence and presence of ORN178 strain in phosphate buffer (pH. 7.2) containing 1.0×10^{-7} M of polymer **A**.

not those of the ORN208 strain, demonstrating specific binding of polymer **A** to FimH protein.

Competition experiments were performed to test the binding ability of α-mannose-bearing polymer **A** to the FimH proteins with respect to free D-mannose in solution. Specifically, free D-mannose was used as a competitor of polymer **A** for FimH proteins in binding experiments by adding free mannose to a mixture of polymer **A** and ORN178 *E. coli* to recover the polymer fluorescence. Free D-mannose at concentrations up to 20 000 times of polymer **A** concentration (1.0×10^{-6} M) has no or little effect on the binding of polymer **A** to bacterial pili. These results suggest that polymer **A** with multivalent display of α-mannopyranosyl groups binds to FimH proteins through cooperative interactions much better than free D-mannose does.

Conclusion

We have described facile, versatile prepolymerization and postpolymerization functionalization approaches to attach α-mannopyranosyl and β-glucopyranosyl residues to poly(*p*-phenylene). These strategies use the reaction of thiols with bromide groups of the monomer and copolymer to form thioether bridges. We believe these approaches could be of great utility in the preparation of fluorescent conjugated glycopolymers, as they should work equally well with any monosaccharide thiol substrate and oligosaccharides. α-Mannose-bearing polymer **A** selectively binds Con A and the pili of the ORN178 strain but not those of the ORN208 strain, which clearly show that fluorescent conjugated glycopoly(*p*-phenylene)s are a simple detection method for protein and bacteria.

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