Sugar-Substituted Poly(*p*-phenyleneethynylene)s: Sensitivity Enhancement toward Lectins and Bacteria

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ABSTRACT: The synthesis of two novel, water-soluble, sugar-substituted poly(*p*-phenyleneethynylene)s and their interactions with concanavalin A (Con A) and bacteria (*Escherichia coli*) are reported, and the issue of sensitivity enhancement is investigated. Both sugar-substituted PPEs (**P5** and **P7**) exhibited strong interactions with Con A with K_{SV} values exceeding 10^8 M^{-1}. The binding constants between the sugar-substituted polymers and Con A were also quantitatively calculated using isothermal titration calorimetry (ITC) resulting in association constants as high as 10^6 M^{-1}. **P5** and **P7** strongly interact with mannose-binding *E. coli*, which led to their aggregation.

Introduction

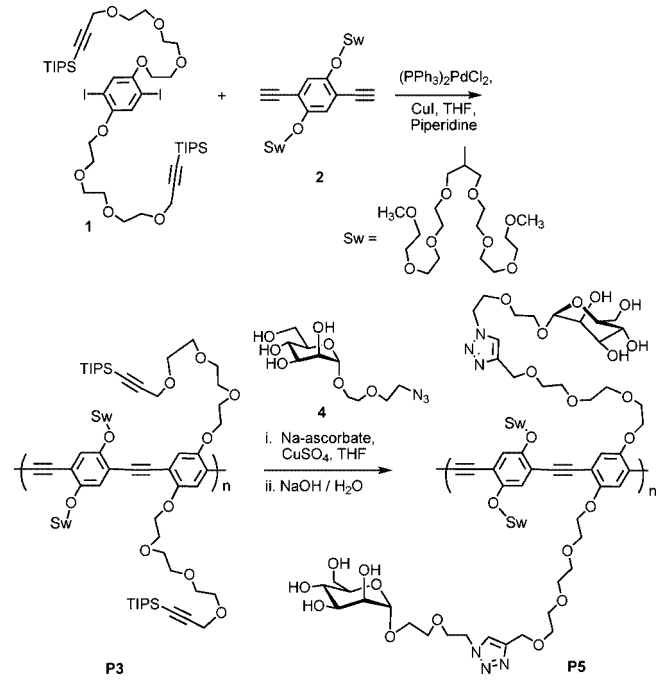
Lectins, proteins which specifically bind sugars, play a crucial role in cell signaling, cell surface recognition, and pathogen docking.[1−3] While lectins such as ricin, *Botulinum* toxin, and the *Escherichia coli* toxin are pathogenic,[4] concanavalin A (Con A), the lectin of the jack bean, is much less toxic and used as proof of principle for novel methods which detect ligand−protein interactions. While Con A binds both to glucose and to mannose, its affinity to mannose is considerably higher. As a consequence, if one wants to construct a tight binding polymeric and polyvalent receptor for Con A, the use of mannosylated macromolecules is advantageous. Agglutination of erythrocytes,[1] surface plasmon resonance of sugar-containing polynorbene derivatives,[5,6] and colorimetric reactions with sugar-coated polydiacetylene vesicles[7,8] are common methods for studying the interaction of lectins and sugars. We[9] and others[10] have previously reported sugar-substituted conjugated polymers for the potential detection of bioterrorist agents and bacteria.[11] While the reported polymers displayed significant binding, it was clearly not sufficient for trace detection of either proteins or microbes. To advance fluorescence-based assays with conjugated polymers as a detection method, one would like to engineer and enhance their sensitivity to such biological analytes. We investigated two possible concepts in this context. One, extending the linker length between the sugar functionality and the conjugated polymer. This should lead to an increased binding between polymer and analyte due to enhanced flexibility and decreased entropic cost. A second concept would be the incorporation of a more complex sugar to increase the innate sensitivity of these conjugated polymers toward lectins and bacteria. We now disclose the synthesis of two sugar-substituted poly(*p*-phenyleneethynylene)s, **P5** and **P7**. These bind to lectins but also stain and/or agglutinate *E. coli* strains.

Results and Discussion

The synthesis of water-soluble sugar-substituted PPEs[12] starts with the preparation of the fundamental building blocks as shown in Schemes 3−5 in the Supporting Information. Palladium-catalyzed coupling of **1** with **2** furnished polymer **P3** which was deprotected *in situ* and subjected to a copper catalyzed 1,3-dipolar cycloaddition with **4** (Scheme 1) resulting in polymer **P5** in 40% yield.[13,14] According to gel permeation chromatography (GPC) vs polystyrene, M_n was 1.1×10^5; **P5** displays a polydispersity index (PDI) of 2.9. Similarly, the palladium-catalyzed coupling of **2** to **6** followed by deacetylation using aqueous sodium hydroxide solution resulted in **P7** in a 31% yield after careful dialysis; **P7** has an M_n of 6.7×10^4 and a PDI of 2.1 respectively (Scheme 2). The PPEs **P5** and **P7** are freely water-soluble, yellow materials which are blue fluorescent in aqueous media. In both cases, the λ_{max} of emission in water is centered at 460 nm. The emission quantum yields in water (ϕ) for **P5** and **P7** are 0.31 and 0.24, respectively.

The quantitative effect of a quencher Q on the emissive intensity F of a fluorophore with the intrinsic fluorescence

Scheme 1. Synthesis of Polymer P5



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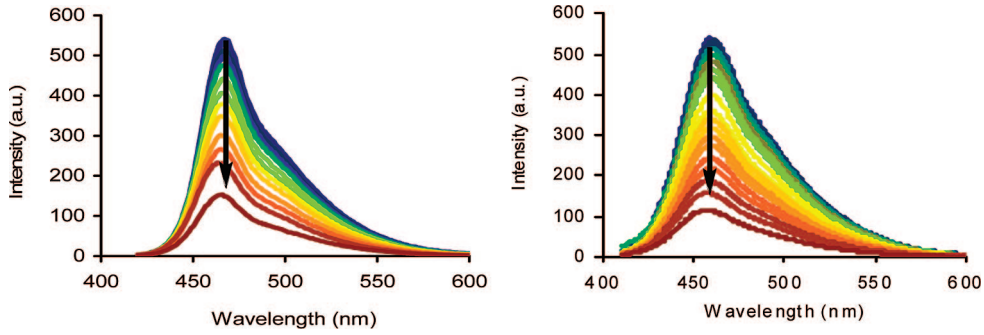


Figure 1. Emission spectra of **P5** (left) and **P7** (right) with increasing concentrations of Con A. Left: Increasing concentrations of Con A (0, 1×10^{-10}, 5×10^{-10}, 1×10^{-9}, 3×10^{-9}, 5×10^{-9}, 8×10^{-9}, 1×10^{-8}, 5×10^{-8}, 1×10^{-7}, 5×10^{-7}, 1×10^{-6}, 5×10^{-6} M). Right: Increasing concentrations of Con A (0, 1×10^{-12}, 5×10^{-12}, 1×10^{-11}, 3×10^{-11}, 5×10^{-11}, 8×10^{-11}, 1×10^{-10}, 5×10^{-10}, 1×10^{-9}, 3×10^{-9}, 5×10^{-9}, 8×10^{-9}, 1×10^{-8}, 5×10^{-8}, 1×10^{-7}, 5×10^{-7}, 1×10^{-6}, 5×10^{-6} M).

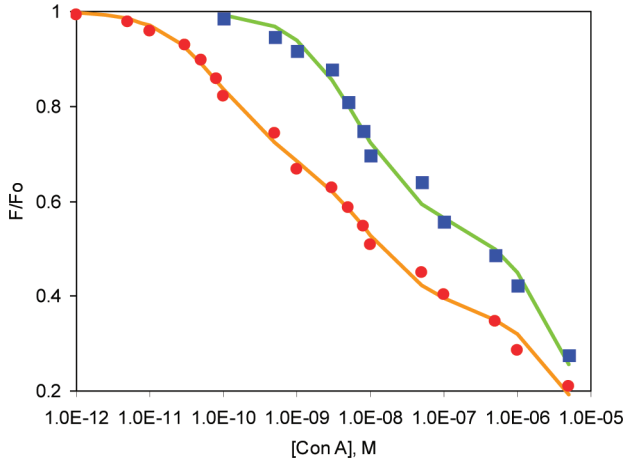
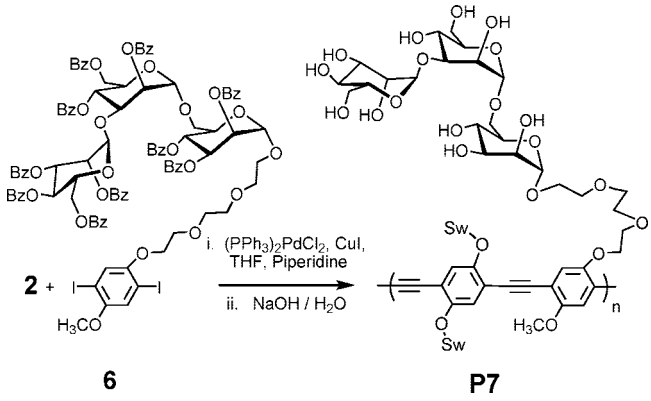


Figure 2. Stern−Volmer plot of emission data obtained from quenching experiments of polymers **P5** and **P7** with Con A. K_{SV_1} and K_{SV_2} are obtained from fit of quenching data to eq 2; (red ●) quenching data points for **P7**; (blue ■) quenching data points for **P5**; (orange line) data fit for **P7**; (green line) data fit for **P5**.

Scheme 2. Synthesis of Polymer P7



intensity F_o is dependent upon the concentration of the quencher Q, [Q]. In the simplest case, a linear relationship, the Stern−Volmer (SV) equation results:[15,16]

$$F_o/F_{[Q]} = 1 + K_{SV}[Q] \quad (1)$$

Quenching of the fluorescence can happen in two fundamentally different ways. If a molecule in its excited state has an emissive lifetime that is >10 ns, molecules of the quencher Q encounter the fluorophore in its excited state and lead to collisional or dynamic quenching. If the emissive lifetimes are short, <1 ns, dynamic quenching is no longer feasible, but static quenching is observed. In static quenching processes, the quencher Q and the fluorophore form a ground state complex, and upon excitation, the preformed complex is efficiently quenched. In the case of PPEs, the emissive lifetimes range from 150 to 400 ps.[17] *As a consequence, collisional quenching processes are present but their contribution is negligible in the fluorescence quenching of PPEs.*

The Stern−Volmer formalism furnishes binding constants between fluorophore and quencher in cases that involve only static quenching. The slope of the Stern−Volmer plot, the Stern−Volmer constant, represents the binding constant between quencher and fluorophore. However, if there are multiple quenching mechanisms, the Stern−Volmer formalism is less straightforward and data analysis yields S−V plots that are nonlinear in appearance and also dependent upon the concentration of the conjugated polymeric fluorophore. In most cases, upward curves result, and the apparent Stern−Volmer constants plummet upon increase of the concentration of the conjugated polymer.[18−20]

In the case of the interaction of sugar-coated conjugated polymers with Con A an unusual behavior is observed in that the slope of the classic Stern−Volmer plot is steep at low [Q] and levels off at higher quencher concentrations, indicating the presence of two binding mechanisms. In addition, we observe that K_{sv} increases upon increasing polymer concentration in the interaction of a negatively charged mannose-substituted PPE with Con A. Such behavior is not reported in the literature; we therefore use a modified version of the Stern−Volmer equation, which addresses the issue of multiple quenching pathways.[21]

$$F_{[Q]}/F_o = \sum_i f_i/(1 + K_{SV_i}[Q]) \quad (2)$$

Such kinetics are provided by eq 2, where f_i, $i = 1, 2, 3, \ldots$, represents the relative fractions of each quenching mechanism ($\sum f_i = 1.0$) and K_{SV_i}, $I = 1, 2, 3, \ldots$, are the respective Stern−Volmer constants.[22]

Known concentrations of Con A were added to solutions of **P5** and **P7** (0.5 μM in phosphate buffered saline) resulting in protein concentrations ranging from the pico- to micromolar range. After each addition of Con A, the fluorescence quenching was monitored by fluorescence spectroscopy (Figure 1) and represented in F/F_o terms (see Figure 2). Using the quenching data from Figure 2 and the two-parameter version of eq 2, we determined by least-squares analysis two equilibrium constants. From the analysis, we obtained a K_{SV_1} for **P5** and **P7** of 1.6×10^8 and 3.2×10^9 M^{-1} and a K_{SV_2} of 2.3×10^5 and 6.9×10^5 M^{-1} with the contribution of each quenching process remaining constant. A better fit was obtained for **P5**, however, than for **P7** (not shown). The trimannose functionality of **P7** may lead to additional quenching mechansims that may cause it to deviate from this two-complex Stern−Volmer equation (eq 2). Thus

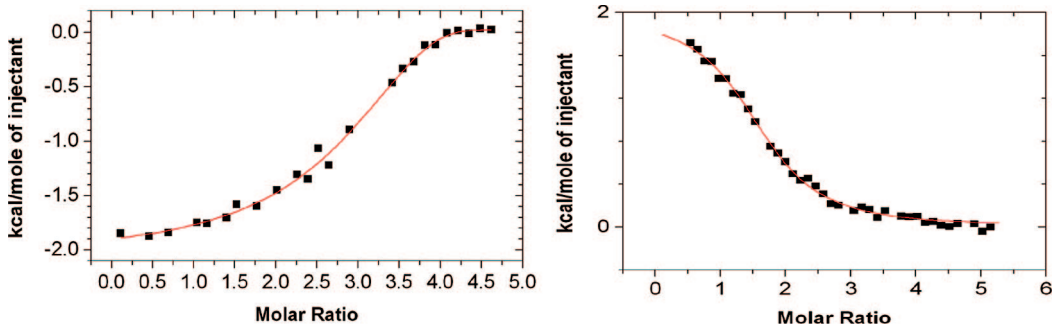


Figure 3. Titration study of **P5** (left) and **P7** (right) with Con A. For each titration, 6 μL of polymer (1 mM in PBS) was injected from a 300 μL syringe at an interval of 5 min into a Con A solution (0.040 mM in PBS) while stirring at 310 rpm.

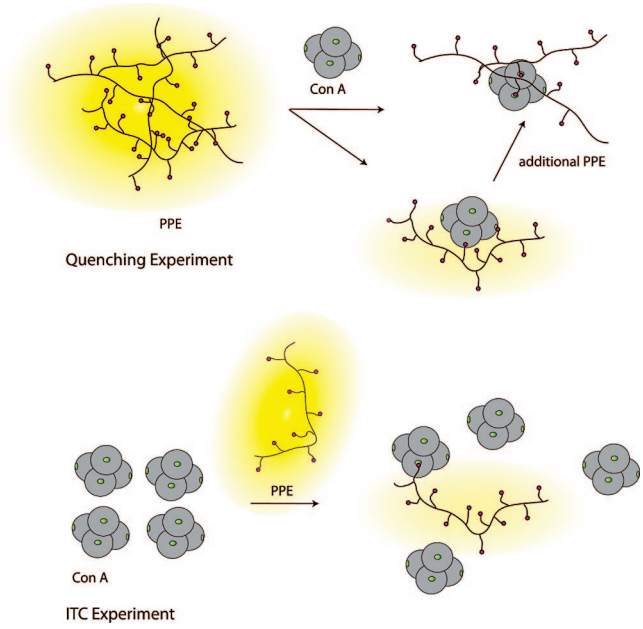


Figure 4. Quenching of the fluorescence of **P5** or **P7** by Con A in the regime of high PPE concentration (as observed by the quenching experiments, top) and at high Con A concentration (as observed in the ITC experiments, bottom).

we used the three parameter fit of eq 2 for **P7**. From this fit, we obtained K_{SV_1} of 1.1×10^{10} M^{-1}, a K_{SV_2} of 1.1×10^{8} M^{-1} and a K_{SV_3} of 1.9×10^{5} M^{-1}. Polymer **P7** contains the trimannose functionality and exhibits a greater affinity for Con A than the mannose-functionalized **P5**, which is reflected by its larger K_{SV} constants and lower limit of detection. This behavior would be expected as the trimannose ligand in **P7** is specific for lectins that display a mannose binding site.

To get a better understanding for the association processes that occur and to quantitatively determine the binding constant, reaction stoichiometry, and thermodynamic profile for the interaction of **P5** and **P7** with Con A, isothermal titration calorimetry (ITC) was used. ITC measures the heat change of a system upon complex formation and provides a thermodynamic profile of the reaction. Since an analyte is titrated into a solution that contains a binding receptor (or receptor into a solution of analyte), K_a and the reaction stoichiometry can directly be obtained.[23] ITC gives a detailed picture of the binding events.

For each titration, the polymer (1 mM in PBS buffer) was injected into a Con A solution (0.040 mM in PBS buffer). As a control, identical samples of each polymer were injected into a PBS buffer solution containing no protein. As expected, the heats of dilution were negligible. The titration data was fitted to a theoretical titration curve using software which was provided by Microcal (One Site Model). The binding constant and thermodynamic parameters were calculated using the equation

$$\Delta G = \Delta H - T\Delta S = -RT \ln K_a \qquad (3)$$

where ΔG, ΔH, and ΔS are the changes in free energy, enthalpy, and entropy, T is the absolute temperature (298 K), $R = 1.98$ cal/(mol K), and K_a is the binding constant.

From the titrations (Figure 3), the association constants for **P5** and **P7** are 1.0×10^5 M^{-1} and 1.6×10^6 M^{-1}, respectively. **P5**, which contained the mannose functionality, exhibited a strong enthalpic contribution (Figure 3, left), while **P7** exhibited a stong entropic contribution (Figure 3, right), which is most likely due to desolvation effects (see Supporting Information). Desolvation effects are not untypical for rigid ligands and have been observed by Brewer et al.[24] As expected, a stronger binding interaction between **P7** and Con A is observed due to the presence of the trimannose functionality.

Figure 4 displays a model that reconciles the large apparent differences between the binding constants obtained from the quenching and the ITC experiments. The quenching experiments are performed in the presence of a large excess of the conjugated polymer. As a consequence, a nonfluorescent, self-quenched 2:1 complex arises. This complex is itself an efficient quencher, extinguishing the fluorescence of further polymer chains that are spatially adjacent to such a quencher complex. As a consequence, the K_{SV_1} values do not represent binding constants, but are better understood as a quenching volume and represent a colligative property of an ensemble. The quenching volume is associated with a Perrin model,[25] in which all quenchers within the quenching volume are assumed to quench with unit efficiency, while quenchers outside are assumed to be ineffective. This is obviously a very crude model when dealing with polymeric quenchers or sensitizers. Leclerc described hyperefficient energy transfer from an ensemble of conjugated polymers to a fluorophore-substituted DNA strand resulting in a similar occurrence of unexpectedly enhanced sensitivity.[26]

On the other hand, the K_{SV_2} values are similar to the binding constants K_a obtained by ITC experiments, in which the polymer was added to Con A. In this case, a 1:1 complex will form. In this interpretation, K_a and K_{SV_2} or K_{SV_3} are binding constants, while K_{SV_1} indicates a *quenching volume* similar to that obtained by the Perrin[25] model; K_{SV_1} therefore would not be a binding constant but an enhancement or amplification factor, describing the ability of the polymer solution to be quenched by a preformed PPE−Con A complex. The increase in the overall K_{SV} with increasing polymer concentration supports this hypothesis. The sensitivity enhancements observed are beyond the association constants obtained from ITC and make functionalized conjugated polymers a powerful tool in potential bioanalytical applications. In conjugated polymers it appears not only

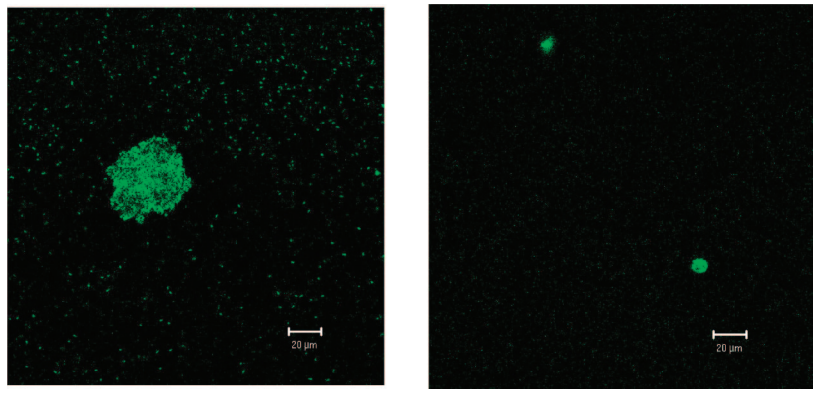


Figure 5. Confocal microscopy image of **P5** with ORN 178 (left) and **P7** with ORN 178 (right). Bacterial cells were grown in LB medium (10 mL) at 37 °C for 16 h to an optical density of 1.0 at 600 nm.

molecular wire and polyvalency effects operate, but hyperefficient energy transfer effects can be engineered to increase their sensitivity toward quenching analytes.

Having successfully examined the binding of **P5** and **P7** with Con A, we decided to investigate their interaction with *E. coli* (strains ORN 178 and ORN 208), which are mannose binding and mutant mannose nonbinding strains, respectively.[27] We were interested to see if the enhanced binding of **P7** over **P5** to Con A would lead to visual differences in their agglutination of the mannose binding *E. coli* strain ORN 178. The strain ORN 208, which has lost its ability to bind to mannose, was chosen as a control. Addition of either **P5** or **P7** to ORN 208 leads to staining of the bacteria, but not to any agglutination. The staining itself is of interest and must be attributed to hydrophobic interactions between the aromatic backbone of the PPE and phenyl rings of aromatic amino acids on the cell wall of the bacteria that are exposed toward the surface. Electrostatic effects are weak or absent, as these polymers are noncharged. However, when **P5** or **P7** is exposed to ORN 178 (Figure 5), agglutination of the bacteria is observed. In the case of **P5** loose aggregates interspersed with single, planktonic cells form, while in the case of **P7** the planktonic bacterial cells have disappeared and instead only dense, fluorescent clusters of bacteria remain. It is remarkable that the increased binding constant of **P7** to Con A is mirrored in the enhanced agglutination of the mannose-binding *E. coli*. It suggests that the mannose binding epitopes on their pili are similar to the mannose-binding receptors of Con A.

In conclusion, we report the synthesis of two sugar-substituted PPEs, **P5** and **P7**, with high sensitivity toward Con A. Increased sensitivity to sugar binding domains results if either the glycosubstituent is placed further away from the conjugated backbone or when more complex sugars are incorporated into the system, reflected in an increased propensity to induce aggregation in *E. coli* when employing **P7**. To determine the binding constant of the lectin−sugar interactions quantitatively, we employed ITC, and a two- or three-component Stern−Volmer equation. Due to the ease of postfunctionalization of our PPEs, this fluorescence based detection system, which exhibits excellent sensitivity toward lectins and bacteria, should easily be adapted toward other analytes and pathogens.

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Supporting Information Available: Synthesis and characterization of all monomers and polymers and details of all experiments performed. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637–674.
(2) (a) Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2755–2794. (b) Kim, I. B.; Erdogan, B.; Wilson, J. N.; Bunz, U. H. F. *Chem. Eur. J.* **2004**, *10*, 6247–6254.
(3) Davis, B. G. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2137–2160.
(4) Deisingh, A. K.; Thompson, M. *Analyst* **2002**, *127*, 567–581.
(5) Kanai, M.; Mortell, K. H.; Kiessling, L. L. *J. Am. Chem. Soc.* **1997**, *119*, 9931–9932.
(6) Smith, E. A.; Thomas, W. D.; Kiessling, L. L.; Corn, R. M. *J. Am. Chem. Soc.* **2003**, *125*, 6140–6148.
(7) Spevak, W.; Foxall, C.; Charych, D. H.; Dasgupta, F.; Nagy, J. O. *J. Med. Chem.* **1996**, *39*, 1018–1020.
(8) Baek, M. G.; Stevens, R. C.; Charych, D. H. *Bioconjugate Chem.* **2000**, *11*, 777–788.
(9) (a) Kim, I. B.; Wilson, J. N.; Bunz, U. H. F. *Chem. Commun.* **2005**, *10*, 1273–1275.
(10) (a) Xue, C. H.; Luo, F. T.; Liu, H. Y. *Macromolecules* **2007**, *40*, 6863–6870. (b) Xue, C. H.; Jog, S. P.; Murthy, P.; Liu, H. Y. *Biomocromolecules* **2006**, *7*, 470–2474. (c) Xue, C. H.; Donuru, V. R. R.; Liu, H. Y. *Macromolecules* **2006**, *39*, 5747–5752.
(11) (a) Disney, M. D.; Zheng, J.; Swager, T. M.; Seeberger, P. H. *J. Am. Chem. Soc.* **2004**, *126*, 13343–13346. (b) McQuade, D. T.; Pullen, A. E.; Swager, T. M. *Chem. Rev.* **2000**, *100*, 2537–2574. (c) Thomas, S. W.; Joly, G. D.; Swager, T. M. *Chem. Rev.* **2007**, *107*, 1339–1386.
(12) (a) Bunz, U. H. F. *Chem. Rev.* **2000**, *100*, 1605–1644. (b) Bunz, U. H. F. *Adv. Polym. Sci.* **2005**, *177*, 1–52.
(13) (a) Englert, B. C.; Bakbak, S.; Bunz, U. H. F. *Macromolecules* **2005**, *38*, 5868–5877. (b) Schweinfurth, D.; Hardcastle, K. I.; Bunz, U. H. F. *Chem. Commun.* **2008**, 2203–2205.
(14) (a) Huisgen, R.; Szeimies, G.; Möbius, L. *Chem. Ber.* **1967**, *100*, 2494–2501. (b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599. (c) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3064. (d) Binder, W. H.; Sachsenhofer, R. *Macromol. Rapid Commun.* **2007**, *28*, 15–54. (e) Wang, Q.; Chittaboina, S.; Barnhill, H. N. *Lett. Org. Chem.* **2005**, *4*, 293–301. (f) Helms, B.; Mynar, J. L.; Hawker, C. J.; Frechet, J. M. J. *J. Am. Chem. Soc.* **2004**, *126*, 15020–15021.
(15) (a) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Kluwer Academic/Plenum Publishers: New York, 1999. (b) Stern, O.; Volmer, M. *Phys. Z.* **1919**, *20*, 183–188.
(16) Zhou, Q.; Swager, T. M. *J. Am. Chem. Soc.* **1995**, *117*, 12593–12602.
(17) (a) Bourdin, E.; Davey, A.; Blau, W.; Delysse, S.; Nunzi, J. M. *Chem. Phys. Lett.* **1997**, *275*, 103–105. (b) Bunz, U. H. F.; Imhof, J. M.; Bly, R. K.; Bangcuyo, C. G.; Rozanski, L.; vandenBout, D. A. *Macromolecules* **2005**, *38*, 5892–5896.
(18) Chen, L. H.; McBranch, D. W.; Wang, H. L.; Helgeson, R.; Wudl, F.; Whitten, D. G. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 12287–12292.
(19) (a) Fan, C. H.; Wang, S.; Hong, J. W.; Bazan, G. C.; Plaxco, K. W.; Heeger, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 6297–6301. (b) Gaylord, B. S.; Wang, S. J.; Heeger, A. J.; Bazan, G. C. *J. Am. Chem. Soc.* **2001**, *123*, 6417–6418. (c) Wang, D. L.; Wang, J.; Moses, D.; Bazan, G. C.; Heeger, A. J.; Park, J. H.; Park, Y. W. *Synth. Met.* **2001**, *119*, 587–588. (d) Wang, J.; Wang, D. L.; Miller, E. K.; Moses, D.; Bazan, G. C.; Heeger, A. J. *Macromolecules* **2000**, *33*, 5153–5158.

(20) (a) Jiang, H.; Zhao, X. Y.; Schanze, K. S. *Langmuir* **2007**, *23*, 9481–9486 (b) Muller, J. G.; Atas, E.; Tan, C.; Schanze, K. S.; Kleiman, V. D. *J. Am. Chem. Soc.* **2006**, *128*, 4007–4016. (c) Tan, C. Y.; Atas, E.; Muller, J. G.; Pinto, M. R.; Kleiman, V. D.; Schanze, K. S. *J. Am. Chem. Soc.* **2004**, *126*, 13685–13694. (d) Tan, C. Y.; Pinto, M. R.; Schanze, K. S. *Chem. Commun.* **2002**, 446–447. (e) Harrison, B. S.; Ramey, M. B.; Reynolds, J. R.; Schanze, K. S. *J. Am. Chem. Soc.* **2000**, *122*, 8561–8562.
(21) Phillips, R. L.; Kim, I. B.; Tolbert, L. M.; Bunz, U. H. F. *J. Am. Chem. Soc.* **2008**, *130*, 6952–6954.
(22) Eftink, M. R. *Top. Fluoresc. Spectrosc.* **1991**, *2*, 53–126.
(23) Jelesarov, I.; Bosshard, H. R. *J. Mol. Recognit.* **1999**, *12*, 3–18.
(24) Dam, T. K.; Roy, R.; Page, D.; Brewer, F. C. *Biochemistry* **2002**, *41*, 1351–1358.
(25) Sun, Y. P.; Wallraff, G. M.; Miller, R. D.; Michl, J. *J. Photochem. Photobiol., A* **1992**, *62*, 333–346.
(26) (a) Ho, H. A.; Boissinot, M.; Bergeron, M. G.; Corbeil, G.; Dore, K.; Boudreau, D.; Leclerc, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 1548–1551. (b) Dore, K.; Leclerc, M.; Boudreau, D. *J. Fluoresc.* **2006**, *16*, 259–265.
(27) Sandra, L.; Spears, P. A.; Havell, E. A.; Hamrick, T. S.; Horton, J. R.; Orndorff, P. E. *J. Bacteriol.* **2001**, *183*, 4099–4102.

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