Layer-by-Layer Poly(phenylene ethynylene) Films on Silica Microspheres for Enhanced Sensory Amplification

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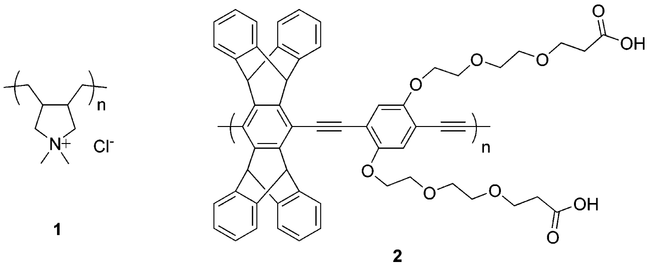
ABSTRACT: Alternating polyelectrolyte "layer-by-layer" deposition has been used to create emissive thin films of a weakly anionic, nonaggregating poly(phenylene ethynylene) on silica microspheres. The resulting particles are smooth and emit fluorescence solely from the outside polymer-coated surface. Suspensions of the coated microspheres show similar fluorescence properties to polymers in solution, but with enhanced sensitivity (up to 200-fold) to nitroaromatic quenchers. These enhancements are attributed to a combination of surface and electronic effects.

Our research group has had a long-standing interest in the application of conjugated polymers such as poly(phenylene ethynylene)s (PPEs) to the detection of trace quantities of analytes of interest.[1] In this field, many of the laboratory demonstrations from our group and others have been focused on the solution response of PPEs to target molecules. However, the use of conjugated polymers in thin-film form has a number of advantages over such solutions in sensory applications. Perhaps the biggest issue is the inherent limitation in energy migration along a one-dimensional structure such as a dissolved PPE in solution. Excited-state electron−hole pairs (excitons) generated in one segment of the polymer chain are free to migrate along the length of the polymer backbone, but the random nature of the exciton "walk" means that the excited state is likely to revisit the same portions of the polymer chain several times during its lifetime. By depositing the polymer as a two-dimensional thin film, excitons are allowed a greater degree of migrational freedom in which the chances of revisiting the same polymer segment are greatly reduced. In an ideal polymer film, this means that a larger number of potential receptor sites can be visited by the exciton, resulting in greater signal amplification and lower detection limits.[2]

Achieving high sensitivity in systems based upon conjugated polymer films requires optimization of the film structure to maximize energy migration while minimizing the deleterious effects of polymer self-aggregation. As part of our program to develop sensory devices based on conjugated polymer-coated glass microstructures, we chose to use silica microspheres as a model substrate for sensory studies. Microspheres are useful from a practical standpoint, since they can be functionalized using standard solid phase synthetic techniques and are easily manipulated through rinsing and filtering procedures. In addition, particles with ferromagnetic cores can be utilized for further separation and have shown use in biomolecular sensing.[3] Microspheres offer an increased surface area for interaction with analytes and have shown utility in creating sensor arrays for the detection of trace analytes,[4] as well as producing photonic structures capable of confining light.[5] Recently, we have also shown that our polymers can achieve enhanced sensitivity in lasing sensors using optical confinement methods,[6] and so the ability to immobilize films of this type is of great interest.

Scheme 1. Structures of PDAC (1) and Poly(phenylene ethynylene) 2



The obvious difficulty in applying spin-coating or alternate physical coating techniques for this purpose led us to consider alternating polyelectrolyte or "layer-by-layer" (LbL) deposition as a method for coating nonplanar surfaces with conjugated polyelectrolytes.[7] The extreme simplicity of the LbL technique—which, being based on repeated immersion of charged substrates into polyelectrolyte solutions, requires equipment no more complex than beakers—has led to its application in the construction of a variety of fluorescent sensory systems, including a pyrene-based fluorescent film sensitive to pH and various metal ions[8] and an unusual LbL structure based on alternating layers of chitosan and CdSe quantum dots coated with anionic headgroups for the detection of the nerve agent paraoxon.[9] When conjugated polymers are deposited using LbL techniques, the silica surfaces can yield a very large refractive index contrast, a useful feature for creating photonic structures.[10] Conjugated polymers have been used in such controlled assemblies to understand multilayer structure[11] and building films for applications in optical devices[12] and sensors.[13] Schanze et al. reported an LbL film based on an anionic PPE bearing short phosphonate-terminated side chains. The quaternary ammonium polycation PDAC (see Scheme 1) and a Zr(IV) species were used as co-polyelectrolytes, and the Zr(IV)-containing film was used in the construction of an electroluminescent device.[14] PPEs containing integrated benzoic acid units have also been used to

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create electroluminescent devices, but in this case the PPE was preaggregated with polyelectrolyte counterions before deposition onto substrates.[15] Our group previously reported LbL films based on a polycationic PPE and a fluorescein-modified polyanion. These assemblies function as polymer-amplified pH sensors in which energy transfer between the PPE and fluorescein is modulated by the pH-sensitive photophysical properties of the fluorescein acceptor.[13a]

In this contribution we report the design and synthesis of a nonaggregating PPE suitable for use in LbL deposition and its application to the preparation of PPE-coated silica microspheres. These microspheres show enhanced fluorescence quenching properties toward nonemissive acceptors vs solution PPEs.

Preliminary experiments involving PPEs modified with hydrophilic, carboxylate-terminated side chains[16] showed that LbL codeposition of these anionic polymers with the strong polycation PDAC (**1**) provided films with well-controlled optical density (directly related to film thickness) but very poor fluorescence properties. This result is unsurprising in light of the known tendency of PPEs to undergo fluorescence-reducing aggregation when cast into thin films. To minimize this effect, we synthesized polymer **2** (M_n = 19 115, PDI = 1.18) containing oligoethylene glycol-linked carboxylic acid groups and a pentiptycene-based monomer unit that discourages π-stacking and is known to provide highly emissive thin films (Scheme 1).[17] LbL films built from deposition of alternating layers of **1** and **2** onto glass slides are brightly fluorescent and show a regular buildup in thickness over several layer pairs, as determined by the film optical density. In these films, **2** displays an absorption maximum of 427 nm, which is blue-shifted relative to the maximum observed in DMF solution (447 nm). This shift reveals that the LbL structure restricts the PPE conformation and prevents the polymer from adopting conformations with the more extended conjugation.

A simple extension of the standard LbL technique provided a method for the deposition of films onto silica microspheres. Aqueous suspensions of microspheres (commercially available as monodisperse preparations ranging in size from 0.64 to 5.0 μm) were treated with base with vigorous shaking, centrifuged and resuspended in deionized water several times, and alternately treated with aqueous solutions of PDAC and DMF solutions of **2**. After washing, the resulting microspheres were bright yellow in color and provided a bright blue-green fluorescence when excited with a hand-held UV lamp. While the optical density of the polymer layers could not be measured by conventional means, zeta-potential measurements indicated a clear alternation of surface charge on the microsphere assemblies after each layer deposition, ranging from strongly positive after PDAC depositions to weakly negative after layers of **2** (Figure 1). The relative strength of the charge after each deposition stage reflects the use of the strong quaternary amine polycation and the weakly polyanionic **2**.

Scanning electron and confocal microscopy confirmed that the microspheres remain smoothly spherical after treatment by the LbL technique. The observation of rings of fluorescence originating from the outside surface of the microspheres (Figure 2) indicates that the PPE is located at the surface, as expected.

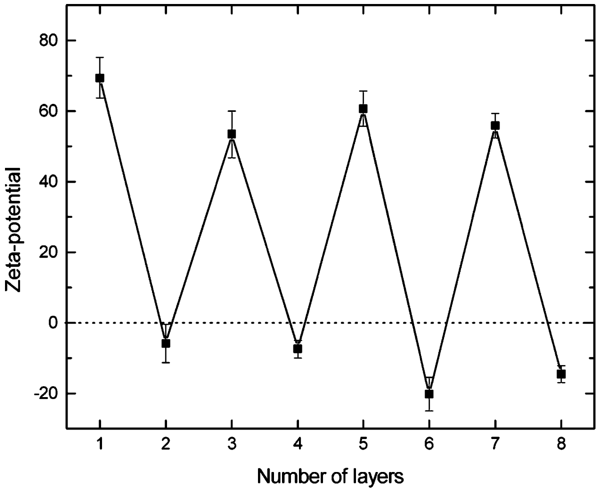


Figure 1. Zeta-potential values for 5.0 μm silica microspheres coated with alternating layers of PDAC (**1**) and a nonaggregating anionic PPE (**2**). Odd layer numbers represent microspheres with a PDAC top layer, while even layer numbers denote microspheres with a PPE top layer. Values shown represent the mean and standard deviation of 10 measurements.

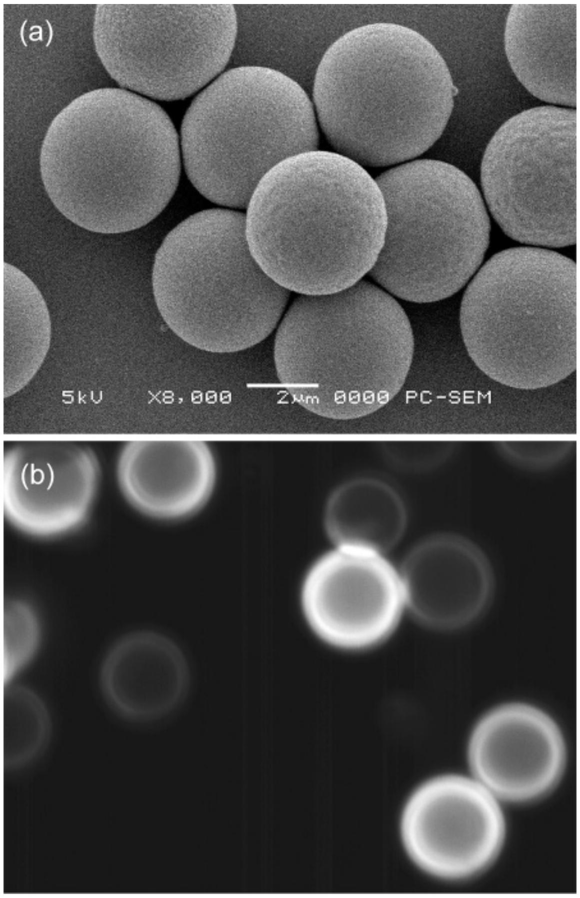


Figure 2. Conjugated polymer-coated microspheres imaged using (a) SEM and (b) confocal microscopy (λ_{ex} = 364 nm).

In addition to the favorable gross structural properties of the PPE-coated microspheres, their fluorescence properties were characterized using fluorescence wavelength-scan confocal microscopy (Figure 3). In contrast to the significant blue shift in the absorption maximum of **2** on moving from DMF solution to LbL films, the fluorescence spectra exhibit nearly identical maxima, thereby indicating that the excited states are mobile in

Table 1. Apparent Stern−Volmer Quenching Constants (K_{SV}), in M^{-1}, for Poly(phenylene ethynylene)s in Solution and Supported as Layer-by-Layer Films on Silica Microspheres[a]

polymer form	4-nitrobenzylamine	LysDNP[b]	BSA-DNP[c]
polymer **3** in solution	$(5.91 \pm 0.08) \times 10^2$	$(1.48 \pm 0.07) \times 10^4$	$(3 \pm 2) \times 10^2$
PDAC/**2** microspheres	$(1.5 \pm 0.4) \times 10^3$	$(1.13 \pm 0.04) \times 10^4$	$(6.6 \pm 0.4) \times 10^4$
(PDAC/**2**)$_2$ microspheres[d]	$(2.69 \pm 0.07) \times 10^3$	$(1.81 \pm 0.04) \times 10^5$	$(9.9 \pm 0.5) \times 10^4$

[a] All K_{SV} values were measured in 0.1 M phosphate-buffered saline, pH 7.3. [b] ϵ-2,4-Dinitrophenyllysine. [c] Bovine serum albumin modified with the dinitrophenyl chromophore. BSA-DNP values are reported on a per DNP chromophore basis. [d] Microspheres coated with two PDAC/**2** layer pairs.

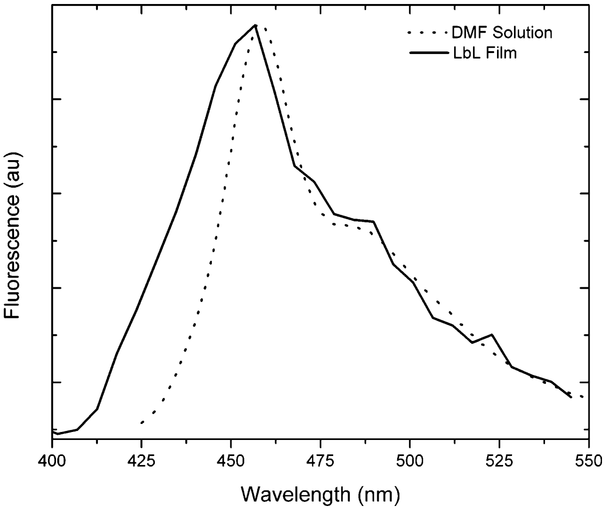


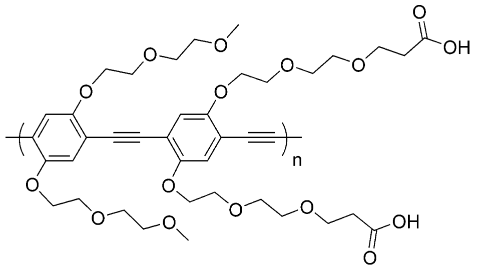
Figure 3. Fluorescence spectra of polymer **2** coated onto a single microsphere (solid line, $\lambda_{ex} = 364$ nm) and in DMF solution (dotted line, $\lambda_{ex} = 405$ nm).

the LbL films and can migrate to minority segments of the polymer having more extended conjugation. This result also demonstrates that LbL-assembled films of **2** are largely immune to the spectral broadening and red shift associated with polymer aggregation.

To evaluate the sensory performance of these microspheres, we compared the response of suspensions of PPE-coated microspheres and PPE solutions to typical fluorescence quenchers in a variety of solvents. Our experiments using microspheres coated with a single PDAC/**2** layer pair and suspended in chloroform showed an apparent Stern−Volmer quenching constant (K_{SV}) of 70 M^{-1} toward 2,4-dinitrotoluene, a value comparable to those observed in polymer solutions. However, when microspheres were suspended in aqueous Tris buffer (pH 7.5) and treated with the electronically similar quencher 3,5-dinitrobenzoic acid, the observed quenching constant rose by 2 orders of magnitude to 9.2×10^3 M^{-1}. This marked increase in apparent K_{SV} on moving to an aqueous environment suggests that hydrophobic effects play an important role in this system and prompted us to investigate microsphere-mediated quenching enhancements in aqueous solution for the biochemically important quencher 2,4-dinitrophenyllysine (LysDNP)[16] both alone and incorporated in bovine serum albumin (BSA), a representative protein. For comparison, we also studied the response of these quenchers to the related water-soluble polymer **3.**[18] The results of these experiments are shown in Table 1.

The most notable trend in the data of Table 1 is that, for all cases, the PPE-coated microsphere systems show larger K_{SV} values than solutions of the electronically comparable polymer **3.** The enhancement provided by a single PDAC/**2** layer pair on a microsphere ranges from about 3-fold for the small, soluble 4-nitrobenzylamine to over 200-fold for BSA-DNP. In the latter case, this large difference may originate from cooperative hydrophobically driven static binding of the protein's multiple DNP groups to the microsphere surface. It is interesting to note that in all cases the microspheres coated with two layer pairs show larger K_{SV} values than those coated with one layer pair. This may be indicative of an energy-migration effect from the underlying layer of PPE to surface PPE in close proximity with the quencher, consistent with the interpenetration of polymer layers observed by Rubner et al.[11c] In addition, quenching efficiency toward single-layer-pair microspheres is significantly larger for BSA-DNP than for LysDNP, while this trend is reversed in the case of two-layer-pair microspheres. This suggests that the smaller analyte LysDNP is able to penetrate farther into the LbL structure, effectively drawing excitons from more remote polymer domains.

Scheme 2. Structure of Water-Soluble Control Polymer 3



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In conclusion, we have described a procedure for preparing highly emissive silica microspheres coated with poly(phenylene ethynylene) using the layer-by-layer assembly method. The resulting microspheres have fluorescence properties comparable to those of the corresponding polymers in solution and show enhanced quenching behavior toward nitroaromatic quenchers. Efforts to apply these results to microsphere-amplified polymer assays are currently underway in our laboratory.

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Supporting Information Available: Experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Swager, T. M. *Acc. Chem. Res.* **1998**, *31*, 201. (b) Wosnick, J. H.; Swager, T. M. *Curr. Opin. Chem. Biol.* **2000**, *4*, 715. (c) Swager, T. M.; Wosnick, J. H. *MRS Bull.* **2002**, 446.

(2) (a) Levitsky, I. A.; Kim, J.; Swager, T. M. *Macromolecules* **2001**, *34*, 2315. (b) Kim, J.; Levitsky, I. A.; McQuade, D. T.; Swager, T. M. *J. Am. Chem. Soc.* **2002**, *124*, 7710.

(3) (a) Graham, D. L.; Ferreira, H.; Bernando, J.; Freitas, P. P.; Cabral, J. M. S. *J. Appl. Phys.* **2002**, *91*, 7786. (b) Nam, J. M.; Stoeva, S. I.; Mirkin, C. A. *J. Am. Chem. Soc.* **2004**, *126*, 5932. (c) Wang, J.; Liu, G.; Munge, B.; Lin, L.; Zhu, Q. *Angew. Chem., Int. Ed.* **2004**, *116*, 2158.

(4) (a) Michael, K. L.; Taylor, L. C.; Schultz, S. L.; Walt, D. R. *Anal. Chem.* **1998**, *70*, 1242. (b) Albert, K. J.; Walt, D. R. *Anal. Chem.* **2000**, *72*, 1947. (c) Goodey, A.; Lavigne, J. J.; Savoy, S. M.; Rodriguez, M. D.; Curey, T.; Tsao, A.; Simmons, G.; Wright, J.; Yoo, S. J.; Sohn, Y.; Anslyn, E. V.; Shear, J. B.; Neikirk, D. P.; McDevitt, J. T. *J. Am. Chem. Soc.* **2001**, *123*, 2559.

(5) Asher, S. A.; Holtz, J.; Liu, L.; Wu, Z. *J. Am. Chem. Soc.* **1994**, *116*, 4997.

(6) Rose, A.; Zhu, Z.; Madigan, C. F.; Swager, T. M.; Bulovic, V. *Nature (London)* **2005**, *434*, 876.

(7) Decher, G. *Science* **1997**, *277*, 1232.

(8) Lee, S.-H.; Kumar, J.; Tripathy, S. K. *Langmuir* **2000**, *16*, 10482.

(9) Constantine, C. A.; Gattás-Asfura, K. M.; Mello, S. V.; Crespo, G.; Rastogi, V.; Cheng, T.-C.; DeFrank, J. J.; Leblanc, R. M. *Langmuir* **2003**, *19*, 9863.

(10) Ho, P. K. H.; Thomas, D. S.; Friend, R. H.; Tessler, N. *Science* **1999**, *285*, 233.

(11) (a) Tarabia, M.; Hong, H.; Davidov, D.; Kirstein, S.; Steitz, R.; Neumann, R.; Avny, Y. *J. Appl. Phys.* **1998**, *83*, 725. (b) Richter, B.; Kirstein, S. *J. Chem. Phys.* **1999**, *111*, 5191. (c) Baur, J. W.; Rubner, M. F.; Reynolds, J. R.; Kim, S. *Langmuir* **1999**, *15*, 6460.

(12) (a) Fou, A. C.; Rubner, M. F. *Macromolecules* **1995**, *28*, 7115. (b) Hong, H.; Davidov, D.; Chayet, H.; Faraggi, E. Z.; Tarabia, M.; Avny, Y.; Neumann, R.; Kirstein, S. *Supramol. Sci.* **1997**, *4*, 67. (c) Ho, P. K. H.; Kim, J. S.; Burroughes, J. H.; Becker, H.; Li, S. F. Y.; Brown, T. M.; Cacialli, F.; Friend, R. H. *Nature (London)* **2000**, *404*, 481.

(13) (a) McQuade, D. T.; Hegedus, A. H.; Swager, T. M. *J. Am. Chem. Soc.* **2000**, *122*, 12389. (b) Wang, X. Y.; Kim, Y. G.; Drew, C.; Ku, B. C.: Kumar, J.; Samuelson, L. A. *Nano Lett.* **2004**, *4*, 331.

(14) Pinto, M. R.; Kristal, B. M.; Schanze, K. S. *Langmuir* **2003**, *19*, 6523.

(15) Thünemann, A. F.; Ruppelt, D. *Langmuir* **2001**, *17*, 5098.

(16) Wosnick, J. H.; Mello, C. M.; Swager, T. M. *J. Am. Chem. Soc.* **2005**, *127*, 3400.

(17) Yang, J.-S.; Swager, T. M. *J. Am. Chem. Soc.* **1998**, *120*, 11864.

(18) Disney, M. D.; Zheng, J.; Swager, T. M.; Seeberger, P. H. *J. Am. Chem. Soc.* **2004**, *126*, 13343.

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