Quantification of Amplified Quenching for Conjugated Polymer Microsphere Systems[†]

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A series of nonaggregating carboxylate-functionalized poly(phenylene ethynylene)s (PPEs) have been synthesized for immobilization via electrostatic adsorption onto Eu^{3+}−polystyrene microspheres with a mean diameter of 0.2 μm. This system is shown to constitute a ratiometric system that measures fluorescence quenching with high fidelity. The fluorescence quenching properties of the polymer-coated particles in response to methyl viologen and a naphthyl-functionalized viologen have been investigated in aqueous solutions to study the influence of electrostatic and hydrophobic interactions with pentiptycene-incorporated as well as macrocycle-containing polymers.

Introduction

The capacity of fluorescent particles to image and monitor biological activity[1,2] has stimulated interest in the development of integrated particle platforms containing conjugated polymers (CPs).[3−8] The extended electronic structure of CPs is highly sensitive to perturbations and provides for an amplified response that has proven useful in numerous sensing applications.[9−11] Additionally, it is recognized that understanding the role of molecular structure and organization in CPs is critical for the creation of more sensitive systems for chemical detection.[12] For biosensing, the molecular design criteria are of paramount importance, and it has proved challenging to design CPS that retain their desired emissive properties while not suffering from nonspecificity in their interaction with analytes.[13,14] With these considerations in mind, we are focusing on constructing particle-based sensory systems that display high fidelity in aqueous environments and demonstrate specificity in a complex chemical/biochemical environment. To this end, we require a reliable and quantitative method for measuring the change in fluorescence upon addition of an electron-deficient quenching species. We employ herein a ratiometric method[15−17] using a noninteracting species as a reference in order to properly quantify the responses as a function of molecular structure. This approach not only allows for precise measurement by providing a standard for comparison[18,19] but also enables direct quantification of quenching species, a beneficial output in any sensory system. In this study, we examine a series of poly(phenylene ethynylene)s (PPE)s, **P1**−**P3**, as films supported on europium-incorporated polystyrene particles ($d = 0.2$ μm) for their fluorescence quenching response toward viologens, which are well-known electron-accepting aromatic molecules. Methyl viologen (**MV^{2+}**) and a naphthyl derivative[20] (**MV^{2+}-nap**) are compared to determine the influence of hydrophobic interactions to promote interactions with the polymer films.[21] **P1** was shown previously to display enhanced quenching on microspheres with 2,4-dinitrotoluene-L-lysine relative to that of a solution-based assay.[22] This enhancement is attributed to greater exciton diffusion lengths and the improved analyte polymer interactions in the solid state. The incorporation of pentiptycene units into the main chain of the polymer yields nonaggregating films with superior emissive properties,[23] and the carboxylic acid moiety allows for electrostatic adsorption onto charged surfaces.[24] We are also interested to investigate the efficacy of molecular receptors[9] under different system conditions, and **P2** and **P3**, the former containing cyclophane receptors, were synthesized to elucidate this role. Polymers **P2** and **P3** have reduced charge densities relative to that of **P1**, and these terpolymers to have a molar ratio of 1:1 pentiptycene unit/comonomer(s) to prevent unfavorable aggregation in the system.[23] This systematic comparative approach was designed to establish

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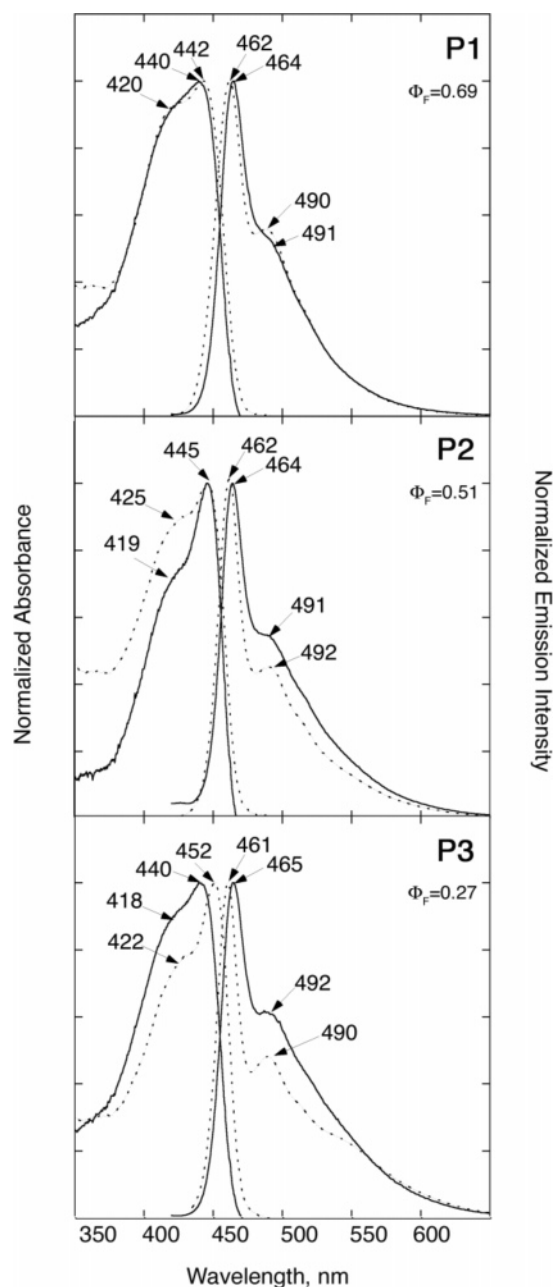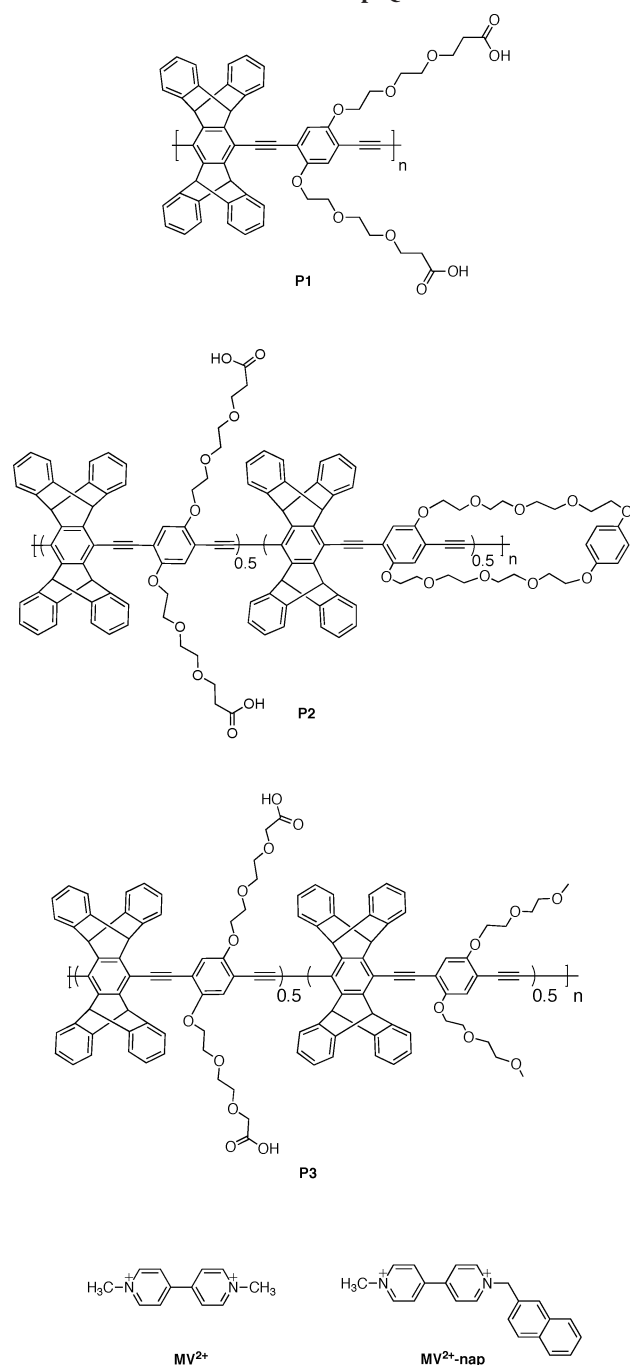
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Figure 1. Absorption and fluorescence spectra of **P1−P3** in DMF (dotted lines) and adsorbed films on glass slides (solid lines). Quantum yields (Φ_F) are in DMF solution referenced to coumarin 6 in EtOH as a standard ($\Phi_F = 0.78$).

a better understanding of the influence of molecular structure on the polymer's performance in biosensing applications.

Results and Discussion

The polymers were synthesized using Pd-catalyzed Sonagashira coupling reactions between respective diacetylene and diiodide monomers in quantitative yield. The carboxylates proved problematic in molecular weight determinations and were converted to dibutylamide groups to provide solubility characteristics that allow analysis by gel permeation chromatography. (See Supporting Information for synthetic and GPC details.) GPC analysis of modified polymers **P1e**, **P2e**, and **P3e** (structures not shown) in THF showed number-average molecular weights of 7 kDa (PDI = 1.4), 8 kDa (PDI = 1.5), and 17 kDa (PDI = 2.2), respectively. The rigid pentiptycene framework prevents

strong interpolymer associations and imparts solubility to **P1−P3** in DMF and mixtures of DMF/water and insolubility in water. Films[25] were formed by electrostatic assembly by immersing glass slides in a 10:90 DMF/water PPE solution (1×10^{-3} M per repeat unit) for 15 min and then subjecting them to thorough rinsing with water and drying in a stream of nitrogen. The slides were initially coated with a trilayer film of poly(ethyleneimine)/ poly(styrene sulfonate)/poly(ethyleneimine) (PEI/PSS/PEI) to minimize defects. The absorption and fluorescence spectra for the polymers in solution and in the immobilized films are shown in Figure 1. The solution and thin film spectra are nearly identical, demonstrating the capability of the bulky pentiptycene substituents to eliminate interchain aggregation.[23]

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(25) The adsorption of P1−P3 on glass slides was monitored by UV−vis absorbance for consistent layer buildup (Supporting Information).
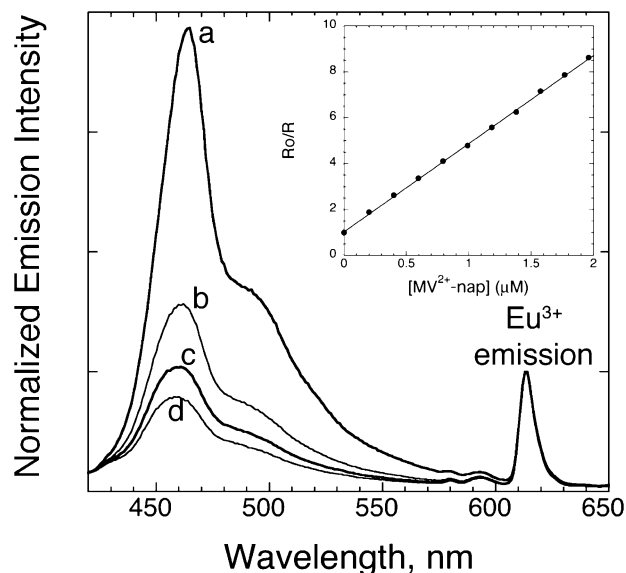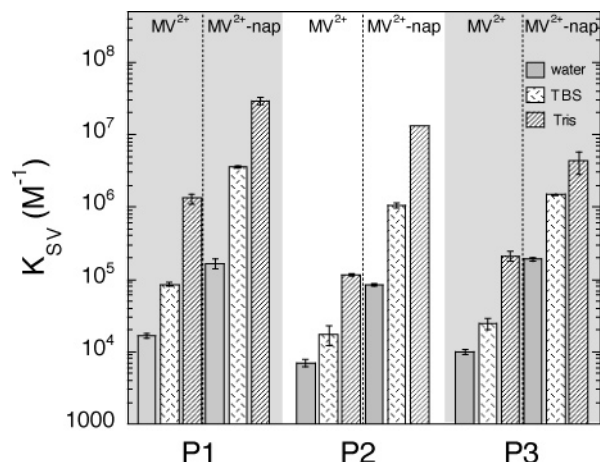
Figure 2. Fluorescence of **P1** particles in Tris buffer (20 mM, pH 7.4), with Eu^{3+} as a reference at 612 nm, in response to (a) 0, (b) 0.06, (c) 0.12, and (d) 0.18 μM additions of **MV^{2+}**-nap. (Inset) Stern−Volmer plot of R_0/R vs [**MV^{2+}**-nap] with a linear best fit (solid line).

The process to produce thin films on particles is similar to that used for the flat substrate; however, because the Eu^{3+}−PS particles are carboxylate-functionalized, a prelayer of PEI is adsorbed before immobilization of **P1**−**P3**. The particles are dispersed in the polymer solution and shaken vigorously for 15 min, followed by centrifugation for 15 min at 14 000 rpm, removal of the supernatant, and two rinsing steps (resuspension, centrifugation, removal of supernatant) in deionized water to ensure complete removal of the excess polymer. The polymer-coated particles are then resuspended (adjusted to a concentration of 0.017% solids) in deionized water, Tris buffer (20 mM, pH 7.4), and Tris-buffered saline (20 mM, pH 7.4; 150 mM NaCl; 5 mM $CaCl_2$) for subsequent quenching experiments. Figure S2 shows a confocal microscopy image of the particles in deionized water, showing uniform fluorescence from the surface.[26]

Excitation at 390 nm allows for direct excitation of both the polymer and the Eu^{3+} incorporated into the PS particles. Figure 2 shows the response of **P1**-coated particles in Tris buffer (20 mM, pH 7.4) toward **MV^{2+}**-nap. Normalization of the spectra to the Eu^{3+} peak at 612 nm accounts for fluctuations in the particle dispersion and for an accurate measure of the quenching response. The magnitude of quenching observed exceeds the diffusion limit and therefore implies that static quenching is operative.[27] A linear best fit yields a Stern−Volmer quenching constant of $(2.9 \pm 0.3) \times 10^7$ M^{-1}.

Figure 3 presents the mean linear best-fit Stern−Volmer constants (K_{SV}) from triplicate measurements for **P1**−**P3** in response to **MV^{2+}** and **MV^{2+}**-nap in deionized water, Tris buffer (20 mM, pH 7.4), and Tris-buffered saline (20 mM, pH 7.4; 150 mM NaCl, 5 mM $CaCl_2$).

Overall, **P1** shows a higher quenching response toward the quenchers. We attribute this to the greater charge density of **P1** relative to that of **P2** and **P3**.[28,29] The differentiation among

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Figure 3. Summary of K_{SV} values for **P1**−**P3** in response to **MV^{2+}** and **MV^{2+}**-nap in deionized water, Tris buffer (Tris, 20 mM, pH 7.4), and Tris-buffered saline (TBS, 20 mM, pH 7.4; 150 mM NaCl, 5 mM $CaCl_2$). Error bars indicate standard errors based on triplicate measurements for each condition.

polymers is most dramatic with the addition of **MV^{2+}**. The responses toward **MV^{2+}**-nap of **P2** and **P3**, however, are not considerably lower than that of **P1**, particularly in water, where electrostatic interactions are strongest. This suggests a dominance of the hydrophobic interactions between the naphthyl rings with the pentiptycene backbone in an aqueous environment relative to the electrostatic interactions of the quaternary amines with the polymer-dependent number of carboxylate side chains. In addition, the π−π stacking interactions with **MV^{2+}**-nap and the polymer may also play a role in the greater observed quenching compared to **MV^{2+}**.

The responses of **P2** and **P3** are very similar for the same conditions. This is in contrast to their behavior in DMF solution, where **P2** ($K_{SV} = (2.9 \pm 0.2) \times 10^6$ M^{-1}) shows greater quenching toward **MV^{2+}** relative to that of **P3** ($K_{SV} = (1.9 \pm 0.4) \times 10^4$ M^{-1}). The DMF solution quenching response of **P2** is even greater than that displayed by **P1** ($K_{SV} = (4.3 \pm 1) \times 10^5$ M^{-1}), illustrating that the macrocycles efficiently bind **MV^{2+}** through hydrogen bonding and π−π interactions[9,30] and thereby increase the overall sensitivity.[9] The formation of a sandwich complex between viologen quenchers and the phenylenediether units of the polymer is a different approach than other microsphere quenching schemes that utilize biomolecular binding, such as streptavidin−biotin interactions,[6] to associate quencher and polymer. In the microsphere system, however, we do not observe any differentiation between the behavior of **P2** and **P3** toward the viologen quenchers. The disparity between solution and film behavior may be due to the film formation process wherein the receptors are geometrically constrained and cannot effectively enhance interactions with the quenchers. Spatially organized films such as the Langmuir−Blodgett type, in which a single monolayer can be deposited, may prove to be a better arrangement for the activity of flexible molecular receptors in aqueous solution.[20]

MV^{2+} and **MV^{2+}**-nap follow similar trends over all of the conditions investigated, with the highest observed quenching in Tris buffer (20 mM, pH 7.4). In this environment, there is a combination of electrostatic and hydrophobic forces. Increased ionic strength shields the electrostatic effects, and Tris-buffered saline displays lower K_{SV} values than does Tris buffer. Interestingly, our results in Tris-buffered saline also reveal a higher degree of quenching than in pure water. This is in direct contrast

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Table 1. Apparent Zeta Potential (in mV) of Polymer-Coated Particles

polymer	water	Tris 20 mM, pH 7.4	Tris-buffered saline
P1	-54.6 ± 0.6	-29.1 ± 0.7	-15.2 ± 1.1
P2	-26.3 ± 0.6	-13.8 ± 1.2	-4.75 ± 1.9
P3	-31.3 ± 0.7	-26.8 ± 1.1	-11.1 ± 1.1

to what has been observed for other conjugated polyelectrolytes in solution,[7,31-34] wherein buffer conditions shield the electrostatic forces and thus reduce the amount of quenching in electrolytes versus that in water.

The zeta potential of the polymer-coated particles (Table 1) indicates the effective charge for each condition. Although water imparts the greatest net negative charge, the observed quenching is the lowest in pure water for all polymers. This further implies that the hydrophobic interactions for these polymers contribute to a greater extent than the electrostatic interactions.[35] This effect produces K_{SV} values for **MV^{2+}**-nap that are more than an order of magnitude higher than for **MV^{2+}**.

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Conclusions

We have synthesized a series of nonaggregating anionic PPEs for adsorption onto Eu^{3+}−polystyrene particles with a mean diameter of 0.2 μm to construct an internally referenced system for measuring the fluorescence quenching of films with high reliability in an aqueous environment. We have been able to measure the response toward quenchers and find that by introducing hydrophobicity with a naphthyl-functionalized viologen (**MV^{2+}**-nap) we can increase the interactions with these polymers, even in environments with high ionic content. We are also investigating how to harness these transduction schemes into even more reliable systems for biosensing in complex environments.

Supporting Information Available: Materials, general methods, detailed synthesis procedure, ^{1}H NMR data of polymers, UV−vis absorbance for layer-by-layer monitoring of **P1**−**P3**, confocal image of **P1**-coated microspheres, and Stern−Volmer plots with a summary table for **MV^{2+}** and **MV^{2+}**-nap. This material is available free of charge via the Internet at http://pubs.acs.org.