Tuning of Superquenching in Layered and Mixed Fluorescent Polyelectrolytes

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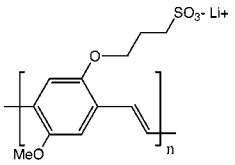
Recent papers have reported on the unusual sensitivity of fluorescent polyelectrolytes to quenching by neutral or oppositely charged quenchers.[1−6] The amplification of quenching sensitivity was first observed with conjugated polymers such as **1**.[1−3] Subsequently, we showed the superquenching extends to formally nonconjugated but "J" aggregated polymers such as the cyanine dye poly-L-lysine derivative **2**.[7] The quenching amplification can be attributed to two factors. The polyelectrolyte shows a strong tendency to associate with the quenchers in aqueous solution similar to the tendency of detergent micelles to "solubilize" many organic molecules. Once bound, a quencher can extinguish fluorescence from a large polymer segment due to a combination of efficient energy migration and exciton delocalization over the polymer.[8−10] The "Stern−Volmer" constants for fluorescence quenching of **1** and **2** are related to the product of the estimated



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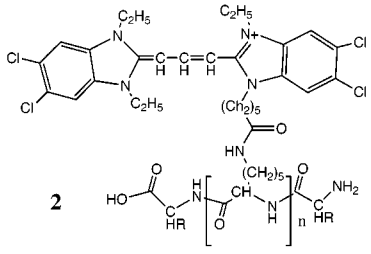

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binding constant and the number of repeat units (n) per polymer.[11] The amplified quenching can be used in sensing applications.[1−3,11] Most previous studies have been carried out in aqueous solutions. Since polymers **1**($n \approx 3000$) and **2**($n \approx 250$) are polyelectrolytes, they can be readily adsorbed as molecularly thin films onto supports such as oppositely charged beads or slides.[12−14] Herein we report a study of polymers **1** and **2** in supported formats and in combination in solution. This study demonstrates that many of the same phenomena observed in solution can also occur in interfacial and supported formats. Moreover, we find that the use of supported or combined formats allows a remarkable tuning and, in some cases, enhancement of superquenching with powerful advantages for biosensing.[1] In aqueous solution the anionic polymer **1** is strongly quenched by cationic electron acceptors such as methyl viologen (MV^{2+}) or the viologen−biotin conjugate $V-B^+$.[1,11]

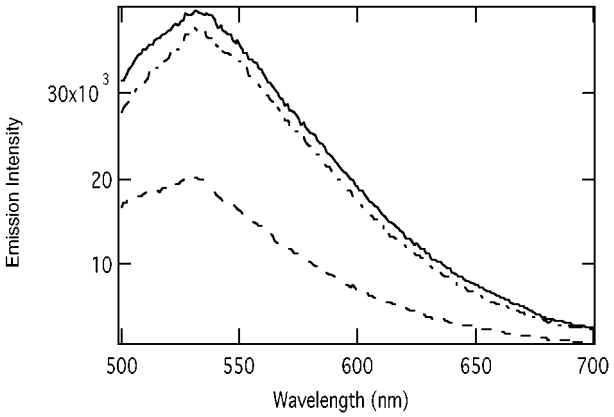

Figure 1. Emission spectra of **1** coated onto 0.2 μm microspheres: unquenched polymer (~1 μM) (solid), $AQ-B^-$ (2.6 μM) quenched polymer (dashed), and Avidin (3.7 μM) quench-recovered polymer (dot−dashed).

The "unquenching" observed when the protein avidin is added to quenched solutions of **1**:$V-B^+$ provides a sensitive quantitative assay for avidin. Similarly, the cationic polymer **2** exhibits superquenching with the anionic anthraquinone-2,6-disulfonate AQ^{2-} and the corresponding conjugate $AQ-B^-$.[7] We have examined the quenching with these quenchers when the polymers are adsorbed onto oppositely charged supports. Polymers **1** and **2** can be coated onto commercial cationic or anionic polystyrene beads.[15] We observe "charge reversal" for the quenching of the supported polymers. Thus, polystyrene-supported **2** is quenched by MV^{2+} but not by the AQ^{2-}, and polystyrene-supported **1** is quenched by AQ^{2-} but not by MV^{2+}. The charge reversal for the supported polymers can extend to biosensing applications. We find polystyrene-supported **1** is quenched by $AQ-B^-$ ($K_{sv} \approx 10^5$ M^{-1}), and addition of avidin results in a regeneration of the polymer fluorescence (Figure 1). Addition of avidin to unquenched polystyrene-supported **1** results in no detectable change in emission, and there is no fluorescence recovery when avidin is added to suspensions of polystyrene-supported **1** that have been quenched by AQ^{2-}. The fluorescence of polystyrene-supported **1** is also quenched by the anionic dye−biotin conjugate $D-B^-$. Here the quenching of polymer fluorescence occurs via energy transfer, and accompanying the quenching is a sensitization of the fluorescence of $D-B^-$. Again a quantitative "unquenching" of the polymer fluorescence can be obtained by adding avidin, indicating displacement of the conjugate from the polymer.

We recently reported that polymer **2** can be adsorbed onto glass slides coated with Laponite clay.[14] The supported polymer exhibits "J" aggregation similar to that observed for the polymer in

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(15) Beads (Pharmacia Source 30Q or 30S) are loaded into a 0.45 μm ultrafiltration tube and washed three times with water under gentle centrifugal force (100*g*) to remove preservative. Beads are resuspended in a polymer solution and then incubated for 1 h at room temperature. Separation of unadsorbed polymer from coated beads is effected by centrifugation for 3 h at 100*g*. Coated beads are then washed three times with water before storing as an aqueous suspension at 4 °C.

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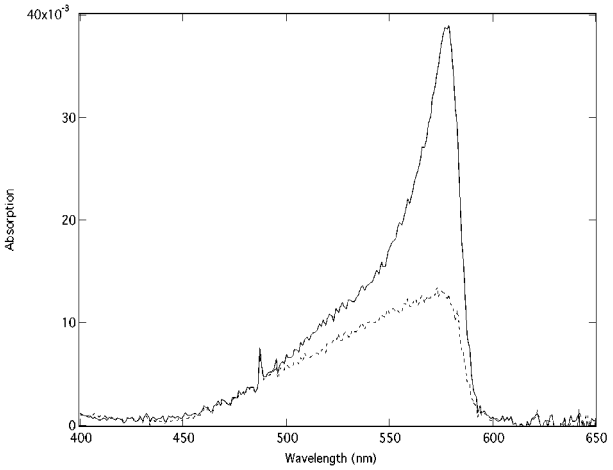


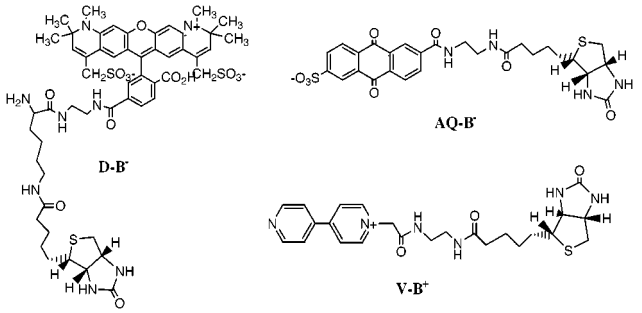
Figure 2. Absorption spectra of 0.17 μM **2** free in solution (solid), and adsorbed onto 2.6 μg of Laponite RDS clay (dashed).

solution. We find that **2** can also be transferred to Laponite clay suspensions in solution; in this case the polymer exhibits an absorption spectrum somewhat blue-shifted compared to the J-aggregate in solution (Figure 2) but still exhibiting chromophore aggregation. The quenching charge reversal may be tuned by adjusting the level of coverage of the polymers on the clay particles.[16] Although from size and charge considerations,[14] we might expect the clay to take up to 20 molecules of polymer **2** per particle, our results indicate that the number of polymer molecules adsorbed per particle may be much lower. Titration of the dye with Laponite suspensions until no additional uptake of polymer occurs results in small changes in light-scattering from dissolved **2** to Laponite-supported **2,** suggesting that each clay particle bears a small number of polymer molecules. Under these conditions, supported **2** exhibits moderately enhanced superquenching by AQ^{2-} ($K_{sv} = 1.1 \times 10^8\ M^{-1}$) compared to aqueous solutions of the same substrate and quencher ($K_{sv} = 7 \times 10^7\ M^{-1}$).[7]

We recently reported that **2** in solution could be quenched by $AQ-B^-$, but not unquenched upon addition of avidin, presumably due to strong association of avidin and **2**.[7] Remarkably, use of **2** in the clay-supported format as described above allows both enhanced superquenching by $AQ-B^-$, as well as quantitative unquenching upon avidin addition. Hence, the use of the supported formats allows us to reduce the sensitivity of the polymers to nonspecific interactions with proteins and at the same time to tune the polymer−quencher interactions. In this way we are able to use $AQ-B^-$ as a quencher for both anionic and cationic polymers in supported formats and to demonstrate biosensing by specific binding and removal of the bioconjugate by its receptor.

When polymers **1** and **2** are mixed together in dilute aqueous solution, there is clear evidence of energy transfer from the higher-energy excited states of **1** to the lower-energy emitting "J" aggregate state of **2**. This interpolymer energy transfer can be observed at very low concentrations and can be reasonably assigned to association between the two polymers. Although the predominant fluorescence of an ensemble formed from an equimolar (in repeat units) mixture of the two polymers is from the "J" aggregate band of **2**, addition of MV^{2+} results in quenching of this fluorescence. The ensemble is also quenched by the addition of AQ^{2-}. Thus, the ensemble can be quenched by both cationic and anionic electron acceptors, and the photophysical properties of the individual polymers are strongly coupled. The quenching by both cations and anions suggests that while the ensemble is overall near neutral, individual regions of each polymer possess sufficient residual charge to strongly bind small counterions and permit superquenching by both types of quenchers. Other work has shown similar energy transfer effects for mixtures of conjugated polyelectrolytes.[17]

While the use of mixtures may provide a means for gaining enhanced quenching, it is also desirable to be able to use mixtures of fluorescent polymers in applications where their individual behavior is retained. We accomplished this by using a format where one of the two polymers is supported and the other remains in solution. Thus, we find that coating of **2** onto Laponite clay followed by addition of **1** in aqueous solution leads to a mixture showing independent behavior of the two polymers both with respect to their fluorescence and quenching. The fluorescence of this mixture with no quenchers added is the simple sum of the fluorescence of each polymer individually: no energy transfer quenching is observed. Addition of MV^{2+} quenches only **1**, while addition of AQ^{2-} to the mixture results in the selective quenching of **2**. This result indicates that supported formats may allow simultaneous sensing of different antigens by several different polymers in the same suspension.



In summary, by layering fluorescent polyelectrolytes onto oppositely charged surfaces, one may tune superquenching effects, eliminate nonspecific interactions, and optimize their use in quantitative bioassays. Also, mixtures of oppositely charged polyelectrolytes offer a means of both charge-tuning and enhanced light harvesting by energy transfer.

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(16) The clay has a higher charge density than the polymer; thus, even fully loaded clay−**2** can be quenched by both MV^{2+} and AQ^{2-}. The selectivity or degree of quenching depends on charge density of the support and polymer and the extent of loading.

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