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Highly Effective Water-Soluble Fluorescence Quenchers of Conjugated Polymer Thin Films in Aqueous Environments

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Conjugated polymers have found excellent use as sensory materials.[1] In particular, these materials have been highly effective in applications that involve sensing inorganic ions and organic small molecules. Viologens are especially potent quenchers of conjugated polymer-based emission. While biosensors utilizing fluorescent conjugated polymers have been developed that take advantage of viologen-based quenchers,[2] these systems invariably rely on Coulombic attractions between water-soluble anionic conjugated polymers (conjugated polyelectrolytes[3]) and cationic viologen quenchers. These systems often display impressive sensitivities, especially when utilized in aqueous solutions of low ionic strength.[4] However, the presence of buffer ions can effectively attenuate the electrostatic attraction between the anionic polymer sensor and cationic quencher.[5] In addition, conjugated polyelectrolyte-based sensors are subject to interference due to nonspecific interactions with charged biological macromolecules.[6] These obstacles represent significant disadvantages that compromise the ability of conjugated polyelectrolytes to act as biosensory materials. Herein we report novel, water-soluble viologen quenchers that bear hydrophobic substituents and describe their effectiveness as quenchers of emission from thin films of hydrophobic, water-insoluble poly(*p*-phenylene ethynylene)s (PPEs) (Figure 1).

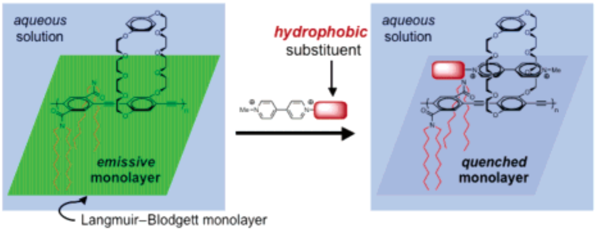

Figure 1. Langmuir–Blodgett monolayer quenching.

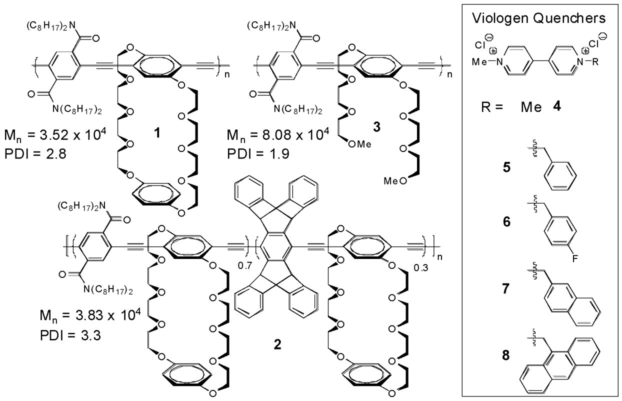

Figure 2. Poly(*p*-phenylene ethynylene)s and viologen quenchers.

To avoid the pitfalls of relying on electrostatic attraction between conjugated polyelectrolytes and cationic viologens and to ultimately arrive at a sensory system that will be amenable to use in solid-state devices, we decided to focus our efforts on water-insoluble, hydrophobic conjugated polymers (Figure 2, Table 1).[7] We have previously shown that both thin films and solutions of neutral, crown ether-containing PPEs are efficiently quenched by cationic viologens in organic solvents.[8,9] However, these sensory materials are much less effective toward detecting viologens in aqueous solution due to strong solvation of the dicationic quencher which dramatically decreases the strength of its binding to neutral, crown ether-functionalized PPEs. This limitation severely compromises the ability of these neutral materials to serve as sensors of biologically relevant macromolecules and processes, applications that often demand performance in aqueous environments. To address this short-

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Table 1. Polymer Photophysical Data[a]

	solution ($CHCl_3$)				LB monolayer			
	abs λ_{max}	em λ_{max}	Φ_F	τ ($\times 10^{-9}$ s)	abs λ_{max}	em λ_{max}	Φ_F	τ ($\times 10^{-9}$ s)
1	423	459	0.44	0.51; 94% 1.22; 6%	432	469	0.12	0.23; 97% 0.95; 3%
2	425	457	0.65	0.53; 96% 1.40; 4%	434	469	0.17	0.24; 97% 1.00; 3%
3	413	456	0.52	0.48; 90% 1.03; 10%	422	479	0.10	0.24; 94% 0.93; 6%

[a] See Supporting Information for details. LB monolayers were prepared on hydrophobic substrates. Absorbance and emission data are provided in nm.

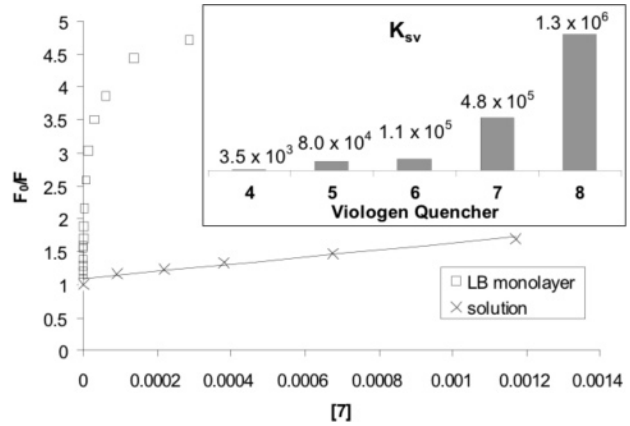

Figure 3. Fluorescence quenching of PPE **1** in solution vs LB monolayer. Measurements were made using PPE **1** and viologen **7**. Solution data were obtained in 20 mM aqueous Tris buffer/THF (9:1) ($K_{SV} = 8.2 \times 10^2$). LB monolayer ($K_{SV} = 4.8 \times 10^5$) data were obtained from a monolayer immersed in an aqueous solution of 20 mM Tris buffer. Inset: quenching response of LB monolayers of PPE **1** to viologens **4**−**8**.

coming, the use of water-soluble, hydrophobically functionalized viologens has been explored (Figure 2).[10]

Initial experiments revealed that quenching of an aqueous solution of **1** with viologens was very inefficient, even when a hydrophobically functionalized viologen was employed. The naphthyl-containing viologen **7**, a promising analyte, quenched emission from **1** in aqueous 20 mM Tris buffer/THF (9:1) solution with a Stern−Volmer constant of only 8.2×10^2 (Figure 3).[11] However, PPE thin films provided significantly improved sensitivity compared to solution-based measurements. Thin film optimization identified Langmuir−Blodgett (LB) monolayers[12] as optimal in terms of sensitivity and stability in 20 mM Tris buffer ($K_{SV} = 4.8 \times 10^5$ for **7** and **1**, Figure 3).[13,14]

The increased sensitivity of LB monolayers over polymer solutions can be attributed to a number of effects. In solution, PPE polymer chains are more isolated and exciton transport is essentially one-dimensional. In LB films, however, facile exciton transport can occur in multiple dimensions as both intra- and interpolymer transport are possible.[12] Higher dimensionality increases the exciton diffusion length as an exciton is less likely to revisit receptor sites. Thus, each film-based excitation is able to sample many more receptors during its lifetime, resulting in greater signal amplification relative to solution phase measurements. LB monolayers are also highly aligned which tends to straighten individual PPE chains and facilitates intrapolymer exciton transport. In addition, LB monolayers are likely to present a highly hydrophobic surface that is superior to a solution phase PPE for interacting with viologens bearing hydrophobic groups such as **5**−**8**.

Examination of the novel viologen quenchers (Figure 2) revealed that the incorporation of hydrophobic substituents has a dramatic effect on quenching efficiency toward LB films of PPE **1** (Figure 3). Dimethyl viologen (**4**) proved to be a very poor quencher, exhibiting a Stern−Volmer constant of only 3.5×10^3. As the hydrophobicity of the additional substituent increased, so too did the observed quenching constants.[15] By simply exchanging a methyl **4** for a benzyl group **5**, the observed K_{SV} was increased by a factor of ∼20. Viologen **6**, which bears a *p*-fluorobenzyl group, provided an additional modest increase in sensitivity. Moving to naphthyl **7** ($K_{SV} = 4.8 \times 10^5$) and anthracenyl **8** ($K_{SV} = 1.3 \times 10^6$) substituents resulted in dramatic increases in sensitivity. These two novel quenchers were more than 2 orders of magnitude more effective than dimethyl viologen **4**. Both **7** and **8** could be detected by crown ether-functionalized PPE films at concentrations below 100 nM. While **8** was slightly better than **7** in terms of observed quenching constants, **7** was kinetically superior. Measurements made with **7** were stable within 5−10 min. However, it took approximately 15−20 min for equilibrium to be reached after addition of aliquots of **8**. For this reason, additional studies focused on naphthyl-functionalized viologen **7**.

The effect of polymer structure has also been studied. The crown ether is a critical component in sensing cationic viologens, even in aqueous solution. Replacing the crown ether in **1** with triethylene glycol substituents **3** ($K_{SV} = 1.4 \times 10^4$) resulted in over a 30-fold drop in sensitivity. The incorporation of a pentiptycene group into the polymer structure **2** provided LB monolayers 2.5 times more sensitive ($K_{SV} = 1.2 \times 10^6$) than **1**. This improvement could be due to increased porosity in LB films of **2** which would allow the hydrophobic naphthyl substituent to better interact with the hydrophobic PPE. Also, PPEs containing iptycene groups are known to produce brighter films with less internal self-quenching compared to films of PPEs without iptycenes. In fact, LB monolayers of **2** ($\Phi_F = 0.17$) are noticeably brighter than those of **1** ($\Phi_F = 0.12$) (Table 1).

Avoiding relying upon electrostatic interactions proved beneficial in terms of generality; quenching of LB films of **2** was largely independent of the nature of the aqueous solution. The quenching constant for **7** toward LB films of **2** was almost the same in 20 mM Tris buffer ($K_{SV} = 1.2 \times 10^6$), 100 mM phosphate buffer ($K_{SV} = 1.3 \times 10^6$), and pure water ($K_{SV} = 1.0 \times 10^6$).

This new sensory system provides high sensitivity in aqueous environments. The sensory response relies on the combination of hydrophobic interactions and the host−guest complexation between a crown ether-functionalized PPE and cationic viologens. Significantly, this system utilizes a hydrophobic polymer film for making measurements in aqueous solution. The thin film nature of this system lends it to potential use in solid-state sensory devices and high throughput screening applications, a significant advantage over solution-based sensors. Future efforts will focus on the incorporation of viologen quenchers into a polymer-bound oligopeptide for use in protease sensing applications.[16]

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Supporting Information Available: Representative experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Crown ethers are excellent hosts for viologens. See: Allwood, B. L.; Shahriari-Zavareh, H.; Stoddart, J. F.; Williams, D. J. *J. Chem. Soc., Chem. Commun.* **1987**, 1064.

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(10) For the synthesis of viologens **5**−**8**, see the Supporting Information.

(11) THF was required to solubilize PPE **1**, as PPE **1** is not soluble in aqueous Tris buffer alone.

(12) For previous studies involving fluorescence quenching of Langmuir−Blodgett films of **1** in organic solvents, see: (a) Levitsky, I. A.; Kim, J.; Swager, T. M. *J. Am. Chem. Soc.* **1999**, *121*, 1466. (b) Levitsky, I. A.; Kim, J.; Swager, T. M. *Macromolecules* **2001**, *34*, 2315. For additional previous studies involving Langmuir−Blodgett films of **3**, see: (c) Kim, J.; Swager, T. M. *Nature (London)* **2001**, *411*, 1030.

(13) Stern−Volmer constants for LB monolayers were obtained from a linear fit of the data at low quencher concentration where the Stern−Volmer plot is linear. As quencher concentration increased, the quenching responses of LB monolayers exhibited downward curvature due to overlapping quenching spheres at high quencher concentration. See the Supporting Information for the linear portion of the Stern−Volmer responses of the LB monolayers.

(14) Thin film optimization revealed no significant dependence of sensitivity or stability upon the nature of the substrate (hydrophobic vs hydrophilic), surface pressure at which the LB films were deposited, or dipping direction. The most important factor was layer thickness; the best stability was achieved with monolayer films. Thicker films proved to be too unstable upon exposure to aqueous buffer as emission from these films trends down with time. See the Supporting Information.

(15) This work was guided by the hypothesis that hydrophobic functionalization of the viologen quencher would decrease the water solubility of the quencher, thereby facilitating desolvation of the quencher upon binding to the hydrophobic polymer. Increased quencher hydrophobicity should increase the driving force for the viologen quencher to come out of aqueous solution and into the hydrophobic polymer thin film.

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