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Amplified Fluorescence Quenching in a Poly(*p*-phenylene)-Based Cationic Polyelectrolyte

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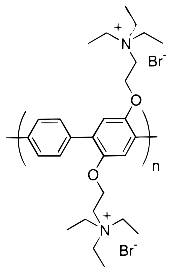
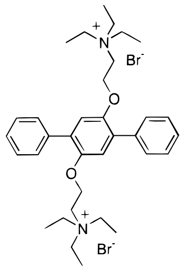
Application of fluorescent conjugated polymers to "amplified" sensing of chemical and biological analytes has received considerable recent attention.[1−8] Fluorescence sensing is amplified by conjugated polymers because of the "molecular wire effect",[1,2] which causes a polymer to be quenched by a considerably lower analyte concentration than its monomer analogue. Most sensor work has been carried out to date using polymers dissolved in an organic solvent;[1,2,5−7] however, several recent reports feature fluorescent thin film sensors that operate by coming into contact with a liquid- or vapor-phase analyte.[3,4] Although these studies demonstrate amplified quenching to allow trace detection of analytes, the systems are limited because the polymers only dissolve in organic solvents. A sensor would be more useful if it operates in an aqueous environment. This issue was addressed by a recent report that demonstrated fluorescence quenching of a water soluble sulfonatoalkoxy poly-(phenylene vinylene) polyanion by methyl viologen at nM concentrations.[8]

In the present contribution we describe fluorescence quenching of the water soluble, poly(*p*-phenylene)-based polycation, P-NEt_3^+ dibromide by several anionic quenchers, including $Ru(phen')_3^{4-}$ and $Fe(CN)_6^{4-}$ in aqueous solution (phen′ = 4,7-bis(4-sulfophenyl)-1,10-phenanthroline). P-NEt_3^+ fluorescence is quenched by these anions with considerably higher efficiency compared to a terphenyl model compound, M-NEt_3^+ dibromide. Amplified quenching of P-NEt_3^+ arises because (1) ion-pairing enhances the concentration of the anionic quencher in the vicinity of the polyelectrolyte, and (2) the high mobility of the $^1\pi,\pi^*$ exciton rapidly brings it into contact with the ion-paired quencher.[9] We also establish that quenching of P-NEt_3^+ by $Ru(phen')_3^{4-}$ occurs via energy transfer by observing the metal complexes' photoluminescence when the excitation light is absorbed mainly by the polymer.



P-NEt_3^+



M-NEt_3^+

Polycation P-NEt_3^+, prepared by Suzuki coupling,[10] was fully characterized by NMR, elemental analysis and GPC which indicated M_n = 12.4 kD (X_n = 28, corresponding to over 50 phenylene rings) and PDI = 1.16.[10] As illustrated in Figure 1a, in aqueous solution P−NEt_3^+ features an absorption at λ_{max} = 330 nm and a strong blue fluorescence with λ_{max} = 408 nm ($\tau \approx$ 600 ps). These features are very similar to those of other PPP-type polymers indicating that the nominal photophysics of P-NEt_3^+ is not strongly influenced by the presence of the quaternary ammonium side-groups.[11−14] The fluorescence of P-NEt_3^+ in aqueous solution is quenched by a variety of anions at a very low concentration. Examples of this effect are illustrated in the Stern−Volmer (SV) plots shown in Figure 2a and 2b, which illustrate quenching of P-NEt_3^+ by $Ru(phen')_3^{4-}$ and $Fe(CN)_6^{4-}$. The SV plots exhibit upward curvature and the quenching efficiencies depend strongly on the polymer concentration.[15] Both of these features indicate that the quencher anions preassociate with P-NEt_3^+ (i.e., quenching is static).[16−18] $Ru(phen')_3^{4-}$ and $Fe(CN)_6^{4-}$ quench the fluorescence of the monomer dication terphenyl model (M-NEt_3^+), also by a static quenching mechanism. However, the quenching of the model is much less efficient—a large stoichiometric excess of the quencher is needed to significantly quench the fluorescence of N-NEt_3^+.[19] The fact that the anions quench M-NEt_3^+ less efficiently than P-NEt_3^+ indicates that the polymer chain amplifies the quenching.

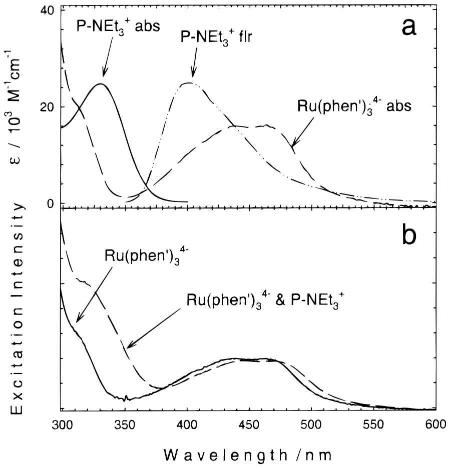


Figure 1. (a) UV−Visible absorption spectra of P−NEt_3^+ (—) and $Ru(phen')_3^{4-}$ (— — —) in H_2O. Fluorescence of P−NEt_3^+ in H_2O (— •• — ••). (b) Emission excitation spectrum with detector set at emission wavelength corresponding to $Ru(phen')_3^{4-}$ MLCT emission (λ_{em} = 610 nm). (solid line): $Ru(phen')_3^{4-}$ only, c = 1 μM; (dashed line): P-NEt_3^+ and $Ru(phen')_3$,$^{4-}$ both at c = 1 μM.

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(15) SV quenching efficiencies obtained by "best fit" linear least-squares are as follows: [P-NEt_3^+] = 1.0 μM, $K_{SV}(Ru(phen')_3^{4-}) = 1.4 \times 10^8$ M^{-1} and $K_{SV}(Fe(CN)_6^{4-}) = 9.3 \times 10^7$ M^{-1}; [P-NEt_3^+] = 10 μM, $K_{SV}(Ru(phen')_3^{4-})$ = 8.0×10^5 M^{-1} and $K_{SV}(Fe(CN)_6^{4-}) = 5.4 \times 10^5$ M^{-1}.
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(18) Static quenching is confirmed by the fact that the fluorescence lifetime of P−NEt_3^+ does not vary with the quencher concentration.

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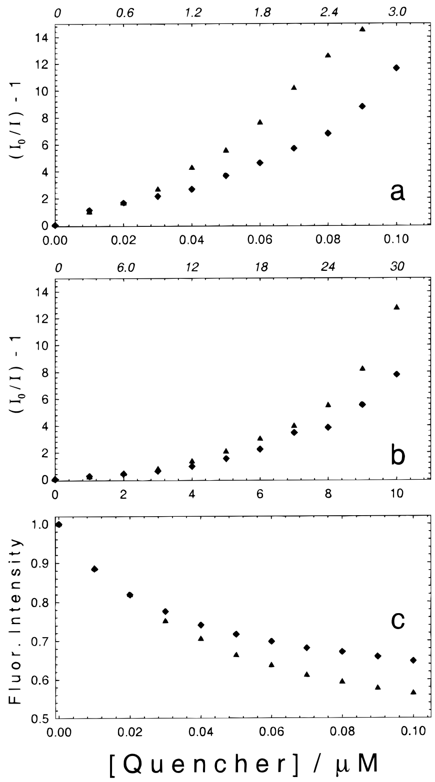

Figure 2. Stern−Volmer quenching plots for P-NEt$_3^+$ in H$_2$O, (▲) Ru(phen′)$_3^{4-}$ and (◆) Fe(CN)$_6^{4-}$. (a) [P-NEt$_3^+$] = 1 μM; (b) [P-NEt$_3^+$] = 10 μM. Italic numbers at the top of (a) and (b) indicate the ratio [quencher]/[polymer chain], where [polymer] = [repeat unit]/X$_n$. (c) Relative fluorescence intensity of adsorbed film of P-NEt$_3^+$ as a function of added quencher to an aqueous solution that is in contact with film, (▲) Ru(phen′)$_3^{4-}$ and (◆) Fe(CN)$_6^{4-}$.

There are several noteworthy features with respect to the P-NEt$_3^+$ quenching data. First, given the short fluorescence lifetime of the polymer, the extraordinary quenching efficiencies clearly indicate that quenching involves an ion-pair between the small molecule tetra-anions and P-NEt$_3^+$.[20] Ru(phen′)$_3^{4-}$ quenches more efficiently than Fe(CN)$_6$,$^{4-}$ suggesting that the larger “amphiphilic” Ru-complex anion associates more strongly with P-NEt$_3^+$. Second, in the quenching studies carried out with [P-NEt$_3^+$] = 1 μM (repeat unit concentration), greater than 90% quenching is observed at [quencher] ≈ 0.08 μM, which corresponds to approximately 12 repeat units per quencher. As such, the quenchers are present at 2:1 [quencher]:[polymer chain] ratio (Figure 2a), and on average binding of one or two quenchers per P-NEt$_3^+$ chain effectively “turns off” the fluorescence of the entire polymer.[21] This implies that in P-NEt$_3^+$, the $^1\pi,\pi^*$ exciton diffuses along the polymer chain to the quencher on a time scale that is rapid compared with its lifetime (600 ps).[9] Time-resolved fluorescence experiments were carried out to determine if the fluorescence decay of P-NEt$_3^+$ in the presence of Ru(phen′)$_3^{4-}$ exhibits a “fast” decay component ($\tau \ll 600$ ps) arising from the polymer-quencher ion pair. However, a fast decay component is not observed, indicating that quenching is instantaneous on the time scale accessible with our instrumentation (200 ps). We conclude that exciton diffusion and quenching occurs with a rate in excess of 10^{10} s^{-1}.

Emission excitation spectroscopy indicates that quenching involves energy transfer from the P-NEt$_3^+$ $^1\pi,\pi^*$ exciton to the triplet metal-to-ligand charge transfer (^{3}MLCT) state of Ru(phen′)$_3^{4-}$. Thus, Figure 1b compares excitation spectra for MLCT emission at 610 nm from a solution containing 1 μM Ru(phen′)$_3^{4-}$ only, and for a solution of Ru(phen′)$_3^{4-}$ and P-NEt$_3^+$ where both the quencher and repeat unit concentration = 1 μM. The significant aspect is that the excitation spectrum of the mixture displays considerably enhanced excitation efficiency in the UV region where P-NEt$_3^+$ absorbs (λ = 300−375 nm). This feature establishes that light absorbed by P-NEt$_3^+$ leads to emission from the MLCT state of Ru(phen′)$_3^{4-}$. In essence the Ru(phen′)$_3^{4-}$ that is ion-paired with the P-NEt$_3^+$ chain acts as a low-energy photoluminescent trap for the highly mobile $^1\pi,\pi^*$ exciton. While the exciton is expected to be very mobile,[1,9,22,23] long-range energy transfer may be facilitated by dipole−dipole (Förster) coupling between the P-NEt$_3^+$ donor and the Ru(phen′)$_3^{4-}$ acceptor. Indeed, a computation based on the spectra and photophysical properties of the two chromophores indicates that the Förster transfer distance (R_o) is ≈ 40 Å.[24] Quenching by Fe(CN)$_6^{4-}$ may also occur by energy transfer, however, since this complex does not photoluminesce it is not possible to confirm that the excited-state complex is produced by quenching.

Although we expected that increasing the P-NEt$_3^+$ concentration would attenuate the efficiency by which the anions quench the polymer, the effect is larger than anticipated. Specifically, Ru(phen′)$_3^{4-}$ and Fe(CN)$_6^{4-}$ quench the polymer approximately 100-fold less efficiently when [P-NEt$_3^+$] = 10 μM compared to that for [P-NEt$_3^+$] = 1 μM (compare Figure 2a and 2b). The decreased quenching efficiency may arise from aggregation of the polycation at higher concentration.

To demonstrate amplified quenching in a solid-state sensor, quenching studies were carried out with P-NEt$_3^+$ thin films.[25,26] Hydrophilic glass slides that had been immersed into an aqueous solution containing P-NEt$_3^+$ (3 mM in repeat units) for 15 min followed by a rinse in distilled water were examined by absorption and photoluminescence spectroscopy. This analysis indicated the presence of a thin adsorbed P-NEt$_3^+$ film (absorption, $\lambda_{\max} = 339$ nm, $A_{\max} = 0.011$; fluorescence, $\lambda_{\max} = 410$ nm).[27] Photoluminescence from the film is quenched strongly when exposed to dilute solutions of Ru(phen′)$_3^{4-}$ or Fe(CN)$_6^{4-}$. Figure 2c illustrates a plot of fluorescence intensity vs amount of quencher added to an aqueous solution that was in contact with an adsorbed film of P-NEt$_3^+$. Detectable quenching is observed upon addition of less than 20 nM of either quencher.

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Supporting Information Available: Description of method used to determine association constant between P-NEt$^+$ and Ru(phen′)$_3^{4-}$ (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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[19] SV quenching efficiencies obtained by “best fit” linear least-squares are as follows: [M-NEt$_3^+$] = 10 μM, K_{SV}(Ru(phen′)$_3^{4-}$) = 24000 M^{-1} and K_{SV}(Fe(CN)$_6^{4-}$) = 8900 M^{-1}.

[20] The K_{SV} values obtained at [P-NEt$_3^+$] = 1 μM imply second-order quenching rate constants that are in excess of the diffusion-controlled rate by a factor of 10^8.

[21] The association constant for Ru(phen′)$_3^{4-}$ binding to P−NEt$_3^+$ has been determined independently ($K_b = 4.6 \times 10^5$ M^{-1}, see Supporting Information). Based on this K_b we estimate that for a solution containing [P-NEt$_3^+$] = 1 μM and [Ru(phen′)$_3^{4-}$] = 80 nM (> 90% fluorescence quenching, see Figure 1a) the concentration of polymer-bound Ru(phen′)$_3^{4-}$ is ≈ 25 nM. Remarkably, the concentration of polymer-bound Ru(phen′)$_3^{4-}$ corresponds closely to the polymer chain concentration, i.e., ([repeat unit]/X$_n$) = (1 μM/28) = 36 nM, which indicates that > 90% quenching occurs when approximately one quencher is bound per chain.

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[27] On the basis of the absorption, we estimate that the film coverage is approximately 2.4×10^{-10} mol-cm^{-2} (repeat unit), which is approximately equal to the coverage expected for a single monolayer of the polymer.