Methodology for Enhancing the Sensitivity of Fluorescent Chemosensors: Energy Migration in Conjugated Polymers

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A common goal in sensory materials is increased sensitivity, which has led to great interest in fluorescence-based chemosensors.[1] However, in spite of progress in instrumentation and materials, there is an ever increasing need for the detection of trace analytes. Increasing the association constant of the recognition (binding) event increases a sensory device's sensitivity. Unfortunately, such an approach to enhanced sensitivity can have the disadvantage of irreversible responses or very long reset times. An additional limitation is that receptors with sufficiently high association constants may not be available. In this report we demonstrate a new method for enhancing the sensitivity of a fluorescence-based chemosensor, which may be used in conjunction with, but does not require, an increased association constant. This method makes use of the special electronic properties of conjugated polymers, which results in an amplified response due to the efficient energy migration to occupied receptor sites.

The electronic properties of conjugated polymers provide unique opportunities for the formation of chemosensory materials. Indeed the sensitivity of their electronic conductivity to structural and chemical perturbations has led to the design of chemoresistive polymers.[2] Conjugated polymers also exhibit unusual photophysical properties, and their emission is often dominated by energy migration to local minima in their band structures. For example, poly(phenyleneethynylene)s have been shown to selectively emit from states associated with anthracene end groups.[3] The emission from electroluminescence devices occurs from regions with greatest conjugation,[4] and the emission from complex ladder polymers can be dominated by defect sites present in low concentration.[5] Thus energy migration appears to be ubiquitous in delocalized polymers and has also been observed in polysilanes which have σ conjugation.[6] Additionally, related processes have been studied in polymers bearing pendant non-interacting chromophores.[7] The physical description of the energy migration process in conjugated materials may involve tightly bound excitons or free carriers, depending upon the material.[8]

To comprehend how energy migration can amplify fluorescence-based sensory events, consider a conjugated polymer with a receptor attached to every repeating unit, wherein the degree of polymerization defines the number of receptor sites, *n*. If energy migration is rapid with respect to the fluorescence lifetime, then the excited state can sample every receptor in the polymer, thereby allowing the occupation of a single binding site to dramatically change the entire emission. In the event that a receptor site is occupied by a quenching agent (i.e., paraquat, PQ^{2+}), the result is an enhanced deactivation (Figure 1). For isolated polymers in solution, the sensitivity may be enhanced by as much as *n* times; however, larger effects may occur in the solid state wherein interpolymer energy transfer may also occur. In principle any polymeric system capable of energy migration can be used. However, for our initial studies we have chosen to investigate poly(phenyleneethynylenes) due to the previous demonstration of efficient energy migration in these materials.[3]

To test this proposal, we made use of bis(*p*-phenylene)-34-crown-10 (BPP) groups, which have been shown by Stoddart to be exceptional receptors for PQ^{2+}, a well-known electron transfer quenching agent.[9] Our synthesis makes extensive use of palladium-catalyzed cross-coupling protocols and is shown in Scheme 1. The diiodide derivative of BPP, **1**, is a key intermediate which allows for efficient preparation of **2**, a highly emissive ethynylene-based fluorophore with a single receptor, and monomer **4**, which when copolymerized with **5** produces polymers **6–8**. The choice of **5** as a comonomer is based upon our qualitative observations that polymers containing this monomer are exceptionally emissive and exhibit high solubility. In the step-growth polymerizations, the molecular weight is determined by the extent of reaction, and at high conversions (>98%) small differences in the conditions and monomer purity can cause large changes in M_n.[10] This sensitivity has produced polymers **6–8** with M_n's (PDI) of 31 100 (1.6), 65 400 (1.6), and 122 500 (1.8), respectively. Additionally we have synthesized **9** (M_n = 268 800, PDI = 2.9) and **3**, which have the same electronic structures as **6–8** and **2**, respectively, but both lack the cyclophane receptors.
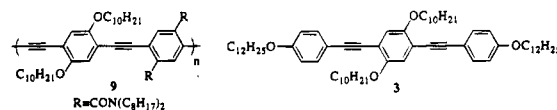
To evaluate these materials we have focused our studies on solution fluorescence measurements. The emission spectra of **6–8** are identical to that of **9** (λ_{max} = 460 nm), indicating that the polymer's band structures are insensitive to the cyclophane structure and the differences in M_n. Electron transfer quenching by PQ^{2+} can occur by either dynamic (collisional) or static (associated complex) processes. A dominance of either process will produce a linear Stern–Volmer relationship ($F_0/F = 1 - K[PQ^{2+}]$), where K is either the dynamic (K_D) or static (K_S) quenching constant.[11] Derivations from this relationship are observed when the two processes are competitive. For PQ^{2+} quenching of **2**, **3**, and **6–9** we observe linear Stern–Volmer relationships (Figure 2). Simple inspection of the plots reveals the large differences between the polymers and their monomeric analogs.

For polymer **9** and **3**, dynamic quenching is dominant. Consequently, no evidence of a charge transfer complex was observed, and the absorbance spectra of **9** (λ_{max} = 426 nm) and **3** (λ_{max} = 368 nm) were unaffected by the addition of PQ^{2+}.

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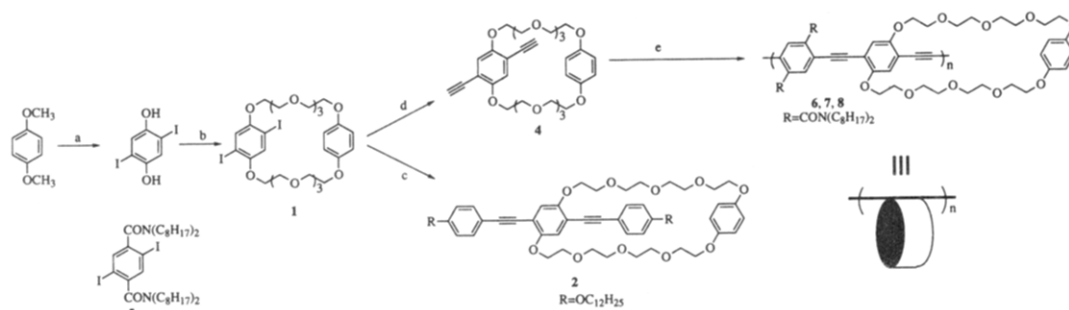
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Scheme 1

^{a} (a) (i) KIO$_3$/I$_2$, HOAc/H$_2$SO$_4$, reflux, 6 h, 85%. (ii) BBr$_3$, −80 °C to room temperature, 48 h, 84%. (b) ClCH$_2$CH$_2$OCH$_2$CH$_2$OH (4 equiv), DMF, K$_2$CO$_3$, 75 °C, 7 days, 70%. (ii) 1,4-bis[2-(2-hydroxyethoxy)ethoxy]benzene bis(tosylate) (1.05 equiv), NaH, THF, reflux, 5 days, 14%. (c) ((*p*-Dodecyloxy)phenyl)acetylene (2 equiv), Pd(PPh$_3$)$_4$ (5%), CuI (5%), (iPr)$_2$NH, 75 °C, 14 h, 80%. (d) (i) TMSA (2.2 equiv), Pd(PPh$_3$)$_2$Cl$_2$ (5%), CuI (5%), (iPr)$_2$NH, 75 °C, 14 h, 80%. (ii) KOH/H$_2$O, THF/MeOH, room temperature, 14 h, 90%. (e) **5** (1.03 equiv), Pd(PPh$_3$)$_4$ (5%), CuI (5%), (iPr)$_2$NH/toluene (30/70), 70 °C, 14 h.
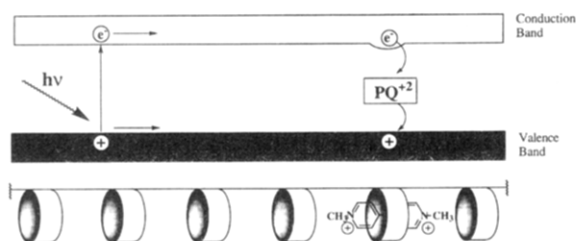

Figure 1. Conceptual illustration of energy migration to a receptor site occupied by PQ^{2+}. The horizontal dimension of the band diagram represents the location along the polymer which is shown schematically. Optical excitation creates an electron−hole pair which migrates throughout the polymer. When the electron encounters a receptor site occupied with a PQ^{2+} group, electron transfer quenching occurs.
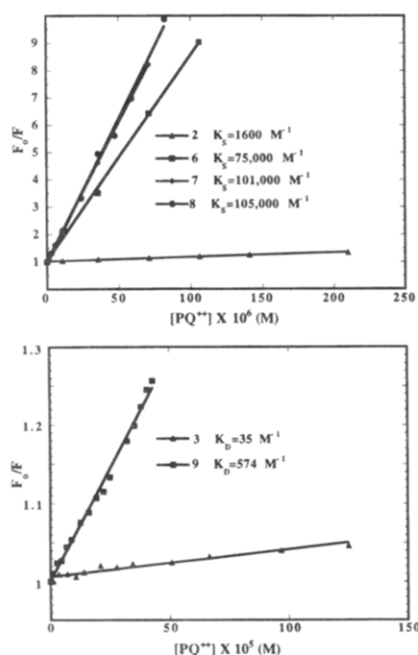

Figure 2. Stern−Volmer plots of **2**, **3**, and **6**−**9**. Note that, for receptor-based materials in the top plot, the range of [PQ^{2+}] is much lower.

The K_D values indicate a greater than 16-fold enhancement in the quenching resulting from the extended electronic structure which produces a mobile delocalized excited state and a larger effective size. It is important to note that this enhancement occurs in spite of the fact that **9**'s lifetime (0.5×10^{-9} s) is considerably shorter than that of **3** (1.2×10^{-9} s). The receptor-based materials are dominated by static quenching. For **6**−**8** the addition of 0.002 M PQ^{2+} shifts the absorption onset from 480 nm (λ_{max} = 426 nm) to 520 nm (λ_{max} = 436), which is

consistent with static quenching by a charge transfer complex. While diffusional quenching is also present for **6**−**8** and **2**, the static quenching is much larger, thereby producing a linear Stern−Volmer relationship with slope K_S. Compound **2** (λ_{max} = 368 nm) also exhibits a new charge transfer absorption (shoulder at 390 nm) in the presence of PQ^{2+} (0.002 M). In agreement with our model, **6**−**8** also show greatly enhanced (47−66-fold increase) K_S values relative to **2**. For static quenching, K_S is simply the association constant with PQ^{2+}. The association constant of PQ^{2+} with **2** is 1600 M^{-1}, and this value is larger than that reported for PQ^{2+} with BPP (K_a = 730 M^{-1}),[12] thereby indicating that the phenylacetylene groups enhance binding.

The degree of enhancement emanating from energy migration is determined by the radiative lifetime and the mobility of the excitations in the polymer. Longer lifetimes and higher mobilities will produce longer average diffusion lengths. For isolated polymers in solution, if this diffusion length exceeds the polymer's length, then an increase in molecular weight will produce greater enhancements. The lower molecular weight analog, **6**, displays a smaller K_S than **7** and **8**, which have comparable values. Hence we conclude that the diffusion length of the excitation exceeds the average length of **6**, but not that of **7** and **8**.

Our results demonstrate a new principle for the amplification of a fluorescence chemosensory event by energy migration in a conjugated polymer. These studies show dramatic enhancements for isolated polymers (in solution), and we expect that solid samples may potentially produce even larger effects. Indeed qualitative investigations on thin films indicate that efficient quenching takes place upon exposure to PQ^{2+}. In the present study we have made use of recognition events which result in fluorescence quenching. However, the energy migration approach to signal amplification is potentially more general, and our continuing studies will focus on extending this concept to other systems wherein binding of the analyte produces a shift in the emission wavelength.

Supporting Information Available: Full experimental details and characterization for the compounds reported (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet. See any current masthead page for ordering information and Internet access instructions.

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