Efficient Quenching of a Guanidinium-Containing Fluorescence Sensor

Wayne N. George,^[a] Mark Giles,^[b] Iain McCulloch,^[a] Joachim H. G. Steinke,^[a] and John C. deMello*^[a]

Conjugated polymers (CPs) have attracted considerable interest owing to their applications in optoelectronic devices such as light-emitting diodes (LEDs),^[1] solar cells,^[2] and thin-film transistors (TFTs).^[3] In recent years there has been increasing interest in exploiting CPs as chemical and biological sensors^[4–7] due to the strong fluorescence quenching they exhibit in the presence of electron deficient species, and their consequent ability to detect trace levels of biological analytes at μ M concentrations and below.^[5,8,9]

The efficiency of the quenching process can be quantified by the Stern–Volmer equation [Eq. (1)] which relates the emission intensity *I* to the quencher concentration [*Q*]:

$$I = \frac{I_0}{1 + k_{\rm SV}[Q]}$$
(1)

where I_0 is the emission intensity in the absence of quencher, and the Stern–Volmer constant k_{sv} is the effective association constant for the complex formed between the fluorophore and the analyte.^[10,11] One of the most widely studied families of fluorescence sensors is that of conjugated polyelectrolytes [CPEs], in which ionically functionalised side chains impart water solubility and provide binding sites for complexation with electron deficient quencher groups. CPEs—and in particular poly(phenylene ethynylenes) [PPEs]—have previously been shown to be highly effective materials for sensing trace quantities of biological materials,^[12–15] exhibiting k_{sy} values that are typically 3-6 orders of magnitude higher than those of their smaller model analogues.^[16] The enhanced sensitivity arises from the tendency of CPEs to form loosely aggregated assemblies in solution, which allows excitons to migrate effectively along and between chains to quenching sites where they decay non-radiatively by an energy or electron transfer process.^[8] Since a single quencher can deactivate the entire assembly, extremely high k_{SV} values can be attained.^[16–19]

CPE sensors studied to date have been based on side-chain polyelectrolytes, in which the ionisable groups are typically attached to alkoxy side-chains and hence are physically isolated from the conjugated backbone.^[14,20] This approach has the ad-

[a]	Dr. W. N. George, Prof. I. McCulloch, Dr. J. H. G. Steinke, Dr. J. C. deMello
	Department of Chemistry
	Imperial College London, South Kensington Campus
	London, SW7 2AZ (UK)
	Fax:(+44) 207 594 5801
	E-mail: j.demello@imperial.ac.uk
[b]	Dr. M. Giles
	Smart Holograms
	291 Cambridge Science Park
	Milton Road, Cambridge, CB4 0WF (UK)
	Supporting information for this article is available on the WWW under
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vantage of enabling a wide palette of existing conjugated polymers to be derivatised with ionic groups but results in poor electronic connectivity between the π -electron system and the complexation site, potentially reducing the quenching effect (especially at low quencher concentrations).

Subsequent attempts to develop improved fluorescence sensors have involved perturbing the electron density along the conjugated chain by introducing electron-donating^[21] or electron-withdrawing^[22] substituents. The inclusion of such groups can enhance electron/energy transfer to a complexed electron-deficient quencher, but their chemical incorporation into the sensor typically requires multistep synthetic routes that significantly limit the scope for further functionalisation of the sensors.^[13,14]

There are potential advantages, in terms of faster electron or energy transfer and improved sensitivity, to incorporating the quenching unit directly into the π system itself but, surprisingly, such an approach does not appear to have been reported in the literature due perhaps to the level of synthetic challenge involved. Herein we report proof-of-principle investigations into a novel small molecule sensor **PE-1** (Figure 1), in which a guanidinium unit is incorporated into the conjugated backbone to impart both water solubility and molecular recognition properties, whilst also maximising electronic connectivity between the fluorophore and a complexed quencher molecule. The behaviour of **PE-1** is compared with that of the structurally related side-chain electrolyte **PE-2**, analogues of which have been widely studied in the literature.

The guanidinium unit is able to form strong ion pairs with oxo-anions such as carboxylates, sulfates and phosphates due



Figure 1. Chemical structures of the two phenylene-ethynylene (PE) based sensors used herein. **PE-1** incorporates a guanidinium unit into the conjugated backbone for solubility and complexation with electron-deficient quenchers, whereas **PE-2** uses ionisable side chains.

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to its planar Y-shaped configuration and its high pK_a of ~12 which ensures protonation over a wide pH range.^[23] We reasoned that connecting the guanidinium motif directly to the chromophore would lead to an enhanced level of quenching relative to **PE-2** due to the direct incorporation of the complexation site into the π -electron system.

The principal steps in the synthesis of **PE-1** are shown in Scheme 1 (see also the Supporting Information). To incorporate the guanidinium unit into **PE-1**, amine **1** was treated with 1,3-bis-(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudo-urea **2** and mercury(II) chloride in DMF following literature procedures,



Scheme 1. Synthesis of **PE-1**. Conditions: i) Et_3N , $HgCl_2$, 2, DMF, 25 °C, 50 h, 68%; ii) K_2CO_3 , MeOH/THF, 25 °C, 4 h, 71%; iii) $Pd(PPh_3)_4$, Cu^{l} , iPr_2NH , 4-iodotoluene, THF, 25 °C, 24 h, 91%; iv) TFA in DCM, 25 °C, 1 h, 59%.

which yielded the guanidinylated derivative **3**.^[24,25] Selective deprotection of the TMS groups produced the corresponding bisalkyne; cross-coupling and cleavage of the Boc groups from **4** gave **PE-1** as a light-yellow crystalline solid in 11% overall yield (7 steps). In addition to providing ready access to **PE-1**, this concise synthetic route has the advantage of generating the orthogonally protected bisalkyne monomer **3** which can be partnered with a wide range of substituted diiodo aryl monomers by palladium cross-coupling chemistry, providing a facile pathway to the synthesis of multiple PPE architectures. The synthesis of **PE-2** has been described previously.^[12]

Figure 2 shows the absorption and emission spectra for **PE-1** and **PE-2** in water. **PE-1** shows a pronounced blue-shift in both absorption and emission relative to **PE-2**. This differs from the usual situation for amine-containing PPEs, in which the absorption and emission bands are usually red-shifted relative to the alkoxy derivatives due to the electron-rich nature of the chain.^[21] The shorter wavelength emission from **PE-1** suggests the overall effect of the guanidinium unit is to reduce the electron density along the PE backbone, consistent with its protonated (and hence electron-withdrawing) state. This is supported by the ¹H NMR data which shows a downfield shift of 0.3 ppm for the proton ortho to the guanidinium motif compared to the equivalent proton in **PE-2**.

The emission spectra of $8 \mu M$ **PE-1** and **PE-2** in water are shown in Figure 3 for varying concentrations of 9,10-anthra-



Figure 2. Normalised absorption and emission spectra in water (pH 7) for 8 μM PE-1 and PE-2. For PE-1 the absorption and emission peaks are at 326 and 376 nm, respectively, whereas for PE-2 they are at 351 and 391 nm.

quinone-2,6-disulfonic acid disodium salt (AQS)—a widely used electron deficient quencher molecule. Strong fluorescence quenching was observed for both sensors, with a slight broadening of the emission spectra and an associated loss of vibronic structure at higher quencher concentrations. Stern–Volmer plots are shown as insets to the main diagrams, with the markers denoting experimental data points and the solid line indicating the optimal fit to the Stern–Volmer relation as described



Figure 3. 8 μ M emission spectra for a) PE-1 and b) PE-2 at various concentrations of the quencher 9,10-anthraquinone-2,6-disulfonic acid disodium salt (AQS) The insets show Stern–Volmer plots using data extracted from the spectra. The intensities in the Stern–Volmer plots are determined from the area beneath the corresponding spectra, and are normalised with respect to the measured emission intensity in the absence of AQS. The markers denote experimental data lines and the solid-lines indicate the optimal fit to the Stern–Volmer relation as outlined in the ESI.

in the ESI. Both sensors exhibited strong quenching in the presence of AQS: **PE-1** had a Stern–Volmer coefficient of $8.9 \times 10^4 \,\text{m}^{-1}$, compared to $4.6 \times 10^4 \,\text{m}^{-1}$ for **PE-2**—the higher value for **PE-1** being consistent with the direct incorporation of the complexation site into the π system.

To verify the generality of the enhanced quenching effect observed with **PE-1**, measurements were also made using a chemically unrelated phosphate-based quencher 4-nitrophenyl phosphate, bis(cyclohexyl-ammonium) salt hydrate (NPP), which has previously been found to be an excellent emission quencher for PPE-based systems.^[12] The emission spectra of 8 μ m **PE-1** and **PE-2** in water are shown in Figure 4 for varying concentrations of NPP. Like AQS, NPP induces strong quenching of **PE-1** and **PE-2** (with only a weak influence on spectral shape), resulting in k_{sv} values of 3.1×10^4 and 1.4×10^4 m⁻¹, respectively.



Figure 4. 8 μ M emission spectra for a) **PE-1** and b) **PE-2** at various concentrations of the quencher 4-nitrophenyl phosphate, bis(cyclohexyl-ammonium) salt hydrate (NPP). The insets show Stern–Volmer plots using data extracted from the spectra.

For both AQS and NPP the quenching efficiency of the guanidinium-containing **PE-1** was approximately twice that of the bis-ammonium derivative **PE-2** (see Figure 5 and Table 1), consistent with the incorporation of the ionic recognition unit into the conjugated backbone and its strong tendency for ion-pair formation with oxo-anions, leading to faster consequent electron transfer to the quencher.^[26–28] The stronger quenching by AQS is presumably a consequence of there being two sulfate binding groups for complexation with guanidinium compared to just one phosphate group in NPP (although this factor may

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Figure 5. Stern–Volmer constants for 8 μ M PE-1 and PE-2 in pure water (pH 7.0) under the influence of the electron-deficient quenchers AQS and NPP. Also see Table 1.

Table 1. Stern–Volmer constants for 8 μM PE-1 and PE-2 in pure water (pH 7.0) under the influence of the electron-deficient quenchers AQS and NPP.

Sensor	Quencher	<i>k</i> _{sv} [м ⁻¹]	Error [m ⁻¹]
PE-1	AQS	8.9×10^4	2.0×10^{3}
PE-1	NPP	3.1×10^4	1.0×10^{3}
PE-2	AQS	4.6×10^4	1.3×10^{3}
PE-2	NPP	1.4×10^4	9.6×10^{1}

be partially offset by the less basic nature of sulfate, which results in weaker individual bond strengths^[23]).

To further compare the behaviour of **PE-1** and **PE-2**, their interaction with simple monovalent ions was investigated by performing additional quenching measurements with the (non-electron-deficient) molecules disodium hydrogen phosphate (DHP) and sodium acetate. **PE-1** underwent unexpectedly strong emission quenching in the presence of DHP, yielding a Stern–Volmer coefficient of $8.2 \times 10^3 \text{ M}^{-1}$ at low DHP concentrations (<0.25 mM), see the Supporting Information.¹ Minimal quenching was observed in the case of sodium acetate, with a k_{sv} value of <5 M^{-1} being observed, indicating a clear selectivity towards phosphates. **PE-2** showed no quenching with either DHP or sodium acetate. The comparative response of **PE-1** and **PE-2** to DHP and (electron-deficient) NPP is shown in Figure 6 and Table 2.

Although **PE-1** was designed to interact strongly with oxoanions, the high degree of quenching observed with DHP is unexpected since neither electron nor energy transfer is expected to occur to DHP. One possible explanation for the quenching effect is the formation of non-emissive aggregates in the presence of DHP. The Y-shaped configuration of the guanidinium unit gives rise to two geometric binding modes for oxo-anions, meaning tetrahedral phosphates like DHP can bind to two guanidinium units at once.^[23] This behaviour was exploited by Nishizawa et al. to develop a pyrene-based guanidinium sensor that self-assembled in the presence of pyrophosphate anions inducing a spectral change that they attributed to excimer emission from the newly π -stacked assembly.^[29] In the present case, it may be that DHP promotes dimer (or

¹ Note, the quenching rate diminished at higher DHP concentrations, causing a deviation from the Stern–Volmer relation.

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Figure 6. Quenching results for 8 μM **PE-1** and **PE-2** in pure water (pH 7.0) under the influence of the traditional electron-deficient quencher NPP and the oxo-anion disodium hydrogen phosphate (DHP). Also see Table 2. The guanidinium-containing **PE-1** undergoes strong quenching in the presence of phosphate ions even with non-electron-deficient molecules such as DHP.

 Table 2. Quenching results for PE-1 and PE-2 in pure water (pH 7.0) under the influence of the traditional electron-deficient quencher NPP and the oxo-anion (DHP).

Sensor	Quencher	k _{sv} [м ⁻¹]	Error [m ⁻¹]
PE-1	NPP	3.1×10^4	1.0×10 ³
PE-1	DHP	8.2×10^3	0.9×10 ³
PE-2	NPP	1.4×10^4	9.6×10 ¹
PE-2	DHP	0.0	-

larger aggregate) formation, leading to a loss of emission by π - π stacking. Whatever the cause, though, it is clear that **PE-1** is an effective sensor for phosphate ions.

In conclusion, we report a novel fluorescent oligo(phenylene ethynylene) **PE-1**, which contains a guanidinium group in direct electronic contact with the conjugated backbone and shows a two-fold gain in sensitivity compared to the structurally related molecule **PE-2**. The results reported herein indicate that the usual strategy of maximising the number of binding sites along a conjugated segment is just one factor for achieving high sensitivity, and that the electronic connectivity between the chromophore unit and the quencher plays a critical role too. Specific recognition groups such as guanidinium and isothiouronium units offer interesting alternatives to the commonly employed ionic water-soluble side groups due to increased selectivity when detecting oxo-anions.

Future work will focus on the synthesis of polymeric analogues of **PE-1**, with a view to signficantly amplifying the fluorescence quenching effect. Initial investigations, using Sonogashira–Hagihara conditions to polymerise the bisalkyne monomer derived from **3** with 1,4-diiodobenzene, yielded a polymer that was highly aggregated in solution. Whilst it was non-emissive in unbuffered aqueous conditions, the addition of a small quantity (0.3 wt%) of the non-ionic surfactant Triton X-100 resulted in strong solution-phase emission that is typical of dialkoxy-PPEs.^[12] We are currently designing derivatives of **3** that will yield less strongly aggregrated polymers and so can act as efficient fluorescence sensors. The results of this work will be reported in due course.

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