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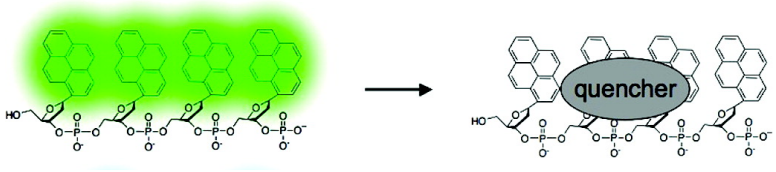

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Efficient Quenching of Oligomeric Fluorophores on a DNA Backbone

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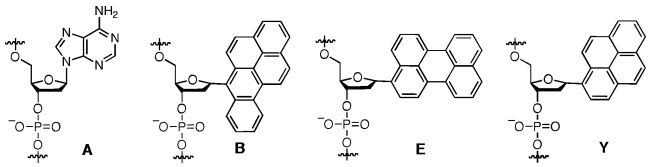
Fluorescence quenching-based strategies have recently become broadly useful in nucleic acid-based biotechnologies. Quencher-fluorophore pairs are widely employed in DNA sequence reporting, and are found in multiple formats such as PNA beacons,[1] varied classes of molecular beacons,[2] quenched autoligation probes,[3] "Scorpion",[4] and "Pacman"[5] probes. Removal of a quencher from a fluorescent label results in an increase in emission, thus yielding a clear and simple signal for the presence of a genetic sequence. However, the sensitivity of such methods depends heavily on the efficiency of quenching, and as a result there has been a substantial amount of research recently on design of new quenchers for existing labels,[6] and on careful matching of specific quenchers with known fluorophores.[7] Even with this optimization, the degree of quenching using discrete labels is often limiting in nucleic acid sequence reporting. Importantly, recent studies of conjugated luminescent polymers have shown exceptionally high quenching efficiencies due to the mobility of the exciton in a given polymer chain.[8] This has led to their recent application in aggregate-based DNA assays.[9] However, such strategies have not found application in labeled nucleic acid probes, possibly because of the large size and inhomogeneity of such polymers and because it would be difficult to conjugate them to synthetic DNA probes.

Here we describe the finding of highly efficient quenching in a different class of oligomeric reporters in which the fluorophores are assembled on a DNA backbone. The molecules are well-defined, relatively small, water-soluble oligomers and are trivial to conjugate to DNA. We find that they can display quenching efficiencies that are unprecedented for discrete organic molecules and rival values previously seen only for conjugated polymeric systems.

We have recently studied these DNA-like fluorophores (oligodeoxyfluorosides (ODFs)) as a new class of reporters and sensors (Chart 1).[10] They display highly diverse and tunable properties depending on length, composition, and sequence. To begin to explore the quenching properties of such fluorophores, we constructed a simple oligomer series containing pyrene nucleoside monomers (see structures) to explore the effect of chain length on the optical properties. It is known that pyrene molecules can interact, depending on their orientation and proximity, exhibiting spectral changes both in the ground state and in the excited state.[11]

On the basis of the structure of DNA itself, the deoxyribose-phosphate backbone of ODFs is expected to bring appended pyrenes into close contact. We found that the absorption spectra of the oligomers **2**−**5** (Figure 1, Table 1) showed clear spectral broadening and a shift to lower energies when compared to the monomer **1**. Thus, neighboring pyrenes in **2**−**5** are not only in close proximity because of restrictions of the DNA backbone, but evidence suggests that they interact electronically in the ground state. Similarly, we found that the pyrene oligomers interact in the excited-state as well: the emission spectrum of **1** shows the expected well-defined vibronic structure with peaks at 376 and 396 nm, while the emission spectra of oligomers **2**−**5** exhibit a broad, featureless peak centered at 490 nm that can be ascribed to emission of excited-state dimers

Chart 1. Structures of Fluorophore Nucleoside Monomers in Sequences **1**−**14**[a]



[a] Specific sequences are detailed in Table 1.

Table 1. Optical Data for Oligomeric Fluorophores **1**−**14** in Water[a]

compd	sequence	abs, λ_{max} (nm)	em, λ_{max} (nm)	Φ_{em}	K_{SV} (M^{-1})	comment
1	Y	**342**, 326	376, 396	0.28	1.9×10^3	
2	Y_2	345, 329	485	0.27	1.3×10^4	
3	SY_3S	345, 329	492	0.21	2.2×10^5	
4	SY_4S	346, 330	490	0.15	5.9×10^5	
5	$S_2Y_8S_2$	348, 332	490	0.16	4.7×10^6	
6	S_2BY	404, **346**	415, 495	-	2.5×10^4	monomer+
					1.1×10^5	exciplex
7	S_3BYE	452, **346**	451, 475	-	2.1×10^5	monomer+
					8.3×10^5	exciplex
8	AYA	**348**, 332	380, 399	0.43	2.4×10^3	
9	$A(YA)_2$	**348**, 332	**380**, 486	0.23	8.5×10^3	
10	$A(YA)_3$	**348**, 332	**380**, 487	0.21	3.7×10^4	
11	$A(YA)_4$	**348**, 332	**381**, 487	0.20	6.1×10^4	
12	Y_4	348, **333**	481	-	1.2×10^7	aggregate
13	Y_6	350, **333**	**465**, 485	-	2.0×10^8	aggregate
14	Y_8	350, **334**	466	-	8.9×10^8	aggregate

[a] All oligomers were prepared as 3′ phosphates; S = THF spacer.

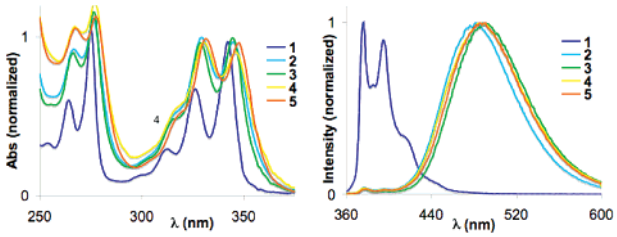


Figure 1. Absorption (left) and emission (right) spectra of **1**−**5**. The significant spectral broadening in the absorption spectra as well as a broad, red-shifted emission of **2**−**5** relative to **1** is indicative of both ground-state and excited-state electronic interactions in the sequences containing multiple pyrene moieties.

(excimers) of pyrene (Figure 1). This interaction is uniform across the series with a ratio of excimer emission to monomer emission of 30:1 or greater (Figure 1). Quantum yields for the molecules in the series ranged from 0.28 for **1** to 0.15 for **4**.

We exposed **1**−**5** in aqueous buffer to methyl viologen (MV), which has previously been employed as a quencher of fluorescent conjugated polymers, DNA labels and pyrene derivatives.[8a,12,14] MV was found to quench the emission of **1**−**5** with Stern−Volmer constants (K_{SV}) ranging from 1.9×10^3 to 4.7×10^6. In the case of the monomeric pyrene, **1**, $K_{SV} = 1.9 \times 10^3$, which is comparable

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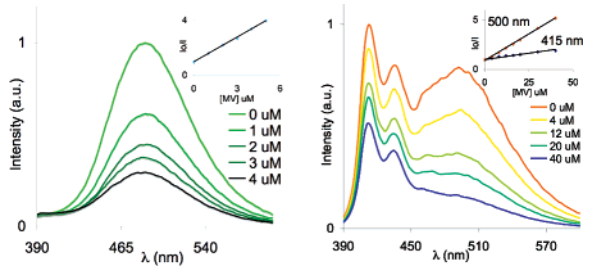

Figure 2. Fluorescence emission quenching of **4** (left) and **6** (right) by methyl viologen at varying concentrations in water. Insets show Stern−Volmer plots. In the case of **6**, greater sensitivity toward quenching is seen for the exciplex emission at 500 nm than for the 415 nm band.

to other small molecule fluorophore−quencher interactions.[13] However, the octamer (**5**) was quenched far more efficiently, with a value ($K_{SV} = 4.7 \times 10^6$) comparable to those for conjugated polymers of much greater length. Thus its quenching is exceptional for a well-defined, relatively small molecule.[8,9,13]

Subsequent experiments showed that efficient quenching is not specific to oligomers of pyrene and can be observed with other π-stacking fluorophores. Sequences containing benzopyrene and perylene in addition to pyrene (**6**,**7**) demonstrated fluorescent excited-state complexes (exciplexes) that were also highly sensitive to quenching by MV (Table 1). Significantly, in cases where both monomer and exciplex emission was present, the delocalized exciplex emission was much more efficiently quenched. For example, in the case of **6** the long wavelength emission resulting from the interaction of benzopyrene and pyrene was quenched 4-fold more efficiently than the emission of benzopyrene alone (Figure 2). This result suggested that the delocalized excited state in these stacked fluorophores is more sensitive to quenching by MV than are the emissions from the single fluorophore components.

To gather more evidence as to the influence of the stacked electronic interactions in this efficient quenching, a second series of oligomers (**8**−**11**) was constructed with adenine interspaced between pyrene residues. These compounds were designed to separate neighboring pyrenes from each other, thus inhibiting ground and excited-state pyrene−pyrene interactions without inhibiting monomeric pyrene emission. As is evident from the absorption spectra, less ground-state electronic coupling is seen in the A-spaced series with the spectra of **8**−**11**. Similarly, the emission spectra in this series show a combination of pyrene monomer and excimer bands, confirming a lessening of excited-state delocalization. Quenching experiments with MV revealed that these adenine-spaced sequences were less efficiently quenched than nonspaced cases, with K_{sv} values approximately one order of magnitude less than for oligomers **2**−**4** containing the same number of pyrenes. For longer pyrene oligomers (e.g., **12**−**14**) containing no solubilizing spacers, evidence for intermolecular aggregation with π-stacking was seen, and interestingly, these were quenched with even higher efficiency than the nonaggregated cases, with exceptionally high K_{sv} values of $\sim 10^7$ to 10^9.

On the basis of these early data we suggest two possible factors that may contribute to this efficient quenching. First, if the excited-state in a multifluorophore stack is mobile (as is the case for conjugated polymers[8]), then collision of a quencher anywhere in the oligomeric fluorophore may cause quenching. This is consistent both with our observation of enhanced quenching of the excimer/exciplex bands over monomer emission and with the long excited states of these delocalized emissions. Second, it is likely that the MV quencher, which is dicationic, has substantial noncovalent binding affinity for these polyanionic molecules; such association may increase the likelihood of quenching as well. Preliminary data at varied ionic strength are supportive of an electrostatic component to the quencher association (see Supporting Information).

In conclusion, we have found that oligomeric DNA-scaffolded fluorophores are highly efficiently quenched by methyl viologen and other known quenchers. We find that quenching efficiency increases with increasing oligomer length and that the efficient quenching can occur with oligomers of varied hydrocarbon fluorophores (pyrene, benzopyrene, or perylene) that display excimer/exciplex excited-state emissions. This suggests that such oligomeric fluorophores may have special utility as reporters and sensors with enhanced sensitivity, both in nucleic acid systems and possibly beyond.

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Supporting Information Available: Characterization data and Stern−Volmer plots for compounds **1**−**14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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