Highly efficient quenching of excimer fluorescence by perylene diimide in DNA†

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Pyrene excimer fluorescence is effectively quenched by non-nucleosidic perylene diimides upon DNA duplex formation.

Fluorescence labeling of biopolymers has become an indispensable tool in many areas of biomedical research.[1–3] The functionalization of proteins, nucleic acids and other biomolecules with fluorescent dyes enables their structural and functional elucidation,[4,5] their cellular localization,[6,7] as well as their quantitation.[3,8,9] The use of two or more fluorescent labels brings additional benefits.[10] Interaction of the dyes through fluorescence resonance energy transfer (FRET)[11] can be used for applications such as signal amplification,[12–15] wavelength shifting[16] or fluorescence quenching.[9] Combinations of fluorophores and quenchers are used in molecular probes that are designed to fluoresce in the presence of a target molecule, whereas in the absence of the target fluorescence is suppressed by the nearby quencher.[9,17] The sensitivity of the probe critically depends on the degree of signal suppression in the absence of the target. Since quenching is often not complete the use of multiple quenchers has been proposed.[18,19] Especially in the case of pyrene excimer fluorescence, entire quenching is difficult to achieve.[19,20] Excimer signals offer advantages, such as a large Stokes-shift and long fluorescence lifetimes.[21] Not surprisingly, interest in excimer-forming oligonucleotide probes is high.[15,22–36] We described the generation of excimers by non-nucleosidic pyrene building blocks in single and double stranded nucleic acids.[25,27,37–39] During our search for building blocks that allow proper control of the fluorescence in such hybrids we found that 3,4,9,10-perylenetetracarboxylic diimide (PDI, building block **E**, see Table 1) is a highly efficient quencher of pyrene excimer fluorescence. PDI and its derivatives have a long-standing history in dye and pigment research. More recently, they have attracted significant interest as electronic materials. Excellent chemical stability and high quantum yields render them attractive for applications in fluorescent materials.[40–42] Since PDI derivatives have a remarkable propensity to form self-assembled aggregates, they have been widely used as building blocks for supramolecular architectures.[41,43,44] PDI-modified oligonucleotide were shown to adopt a variety of structures, including duplex, hairpins, triplex, quadruplex, as well as larger structures.[30,43,45–48] It was noted that fluorescence of PDI is significantly reduced upon attachment to DNA.[45,49,50] However, perylene diimide has not been described as a fluorescence quencher. Here we report its use as a highly potent quencher of pyrene excimer fluorescence.

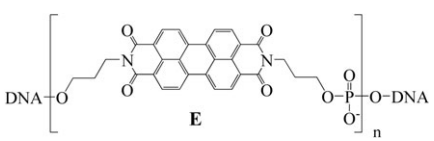
The PDI-building block was prepared according to the literature procedure.[48] Modified oligonucleotides were prepared by conventional means (ESI†).[51] Melting temperatures (T_m) are shown in Table 1. In comparison to the unmodified duplex **1**·**2**, hybrid **3**·**4** containing one PDI in each strand showed a remarkable increase in hybrid stability (ΔT_m = 11.3 °C), which is in agreement with the literature and can be attributed to favorable stacking properties of the perylene diimides.[49] Addition of a second PDI in each strand (**5**·**6**) resulted in a further stabilization (ΔT_m = 13.5 °C).

Fluorescence spectra of single strands **3**–**6**, as well as hybrids **3**·**4** and **5**·**6** showed no significant emission (ESI), rendering PDI rather uninteresting as a fluorescent building block in the current context. Circular dichroism (CD) spectra of both hybrids are consistent with an overall *B*-DNA conformation. For both hybrids, **3**·**4** and **5**·**6**, exciton coupled CD (EC-CD)[52,53] was observed (ESI). Bisignate signals for the perylene band are centered at 531 nm (A = 95)[52] and 525 nm (A = 77), revealing a twisted arrangement of the PDI units.

Analysis of hetero-hybrids (*i.e.* composed of a perylene- and a pyrene-modified strand, Table 2) led to a unexpected finding: duplex formation is accompanied by highly efficient quenching of the pyrene fluorescence (Fig. 1). The excimer signal of the pyrenes[38,54–57] in **7** is completely suppressed by oligomer **6**, which has two PDI building blocks opposite to the pyrenes. It

Table 1 T_m values of DNA hybrids containing two or four PDI units

Oligo	Duplex	T_m[a]/°C	ΔT_m/°C
1	(5′) AGC TCG GTC ATC GAG AGT GCA	72.8	—
2	(3′) TCG AGC CAG TAG CTC TCA CGT		
3	(5′) AGC TCG GTC A**E**C GAG AGT GCA	84.1	+11.3
4	(3′) TCG AGC CAG T**E**G CTC TCA CGT		
5	(5′) AGC TCG GTC **EE**C GAG AGT GCA	86.3	+13.5
6	(3′) TCG AGC CAG **EE**G CTC TCA CGT		



E

[a] *Conditions*: 1.0 μM oligonucleotide (each strand), 10 mM phosphate buffer (pH 7.4) and 100 mM NaCl.

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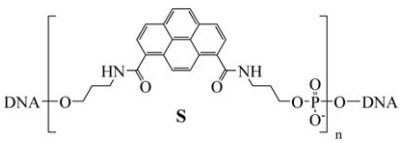
Table 2 Quenching of pyrene excimer fluorescence by perylene diimide

Oligo	Duplex	T_m[a]/°C	Q[b] (%)
7	(5′) AGC TCG GTC **SSC** GAG AGT GCA		
7 **2**	(5′) AGC TCG GTC **SSC** GAG AGT GCA (3′) TCG AGC CAG TAG CTC TCA CGT	64.6	46
7 **4**	(5′) AGC TCG GTC **SSC** GAG AGT GCA (3′) TCG AGC CAG **TEG** CTC TCA CGT	73.5	56
7 **6**	(5′) AGC TCG GTC **SSC** GAG AGT GCA (3′) TCG AGC CAG **EEG** CTC TCA CGT	75.5	98.6



[a] *Conditions*: see Table 1. [b] Quenching effect, measured as the ratio of excimer signals: $Q = 100\{1 - [F(\text{hybrid})/F(\text{single strand})]\}$.

is important to note that the mixed hybrids **7·4** and **7·6** possess high thermal stabilities. The stabilizing effect of a PDI building block is comparable to that found in the hybrids containing only PDI modifications (see Table 1).

The observed quenching effect cannot simply be explained by the nearby located guanines, which are known for their quenching properties.[58] The unmodified oligonucleotide **2**, containing two guanines, has a quenching effect in the order of 46% (or 23% per guanine base). We assume that the quenching is a result of the formation of a non-fluorescent complex between the pyrene and PDI units and/or energy transfer from pyrene to PDI. Due to a good spectral overlap of PDI absorbance and pyrene excimer emission (ESI†), energy transfer can readily take place. On the other hand, a single PDI unit in the complementary strand (hybrid **7·4**) has a moderate quenching effect (~10% increase compared to the unmodified complementary strand **2**). Thus, the quenching caused by oligomer **6** is not simply explained by the sum of the individual contributions. Reduction of the excimer signal can take place by electronic quenching of the excited monomer or of the excimer. Additionally, it may be due to inhibition of excited dimer formation by steric interference with the PDI units. An interesting observation in this regard is the change in exciton chirality in the CD spectra (Fig. 2). While the PDI-modified hybrids **3·4** and **5·6** both show a negative chirality, the couplet of the perylene signal in the mixed hybrid **7·6** is of opposite chirality. The switch indicates a fundamental change in the way the perylenes interact. In addition, the amplitude of the CD signal is significantly reduced in the mixed hybrid ($A = 30$ in **7·6** *vs.* 95 and 77 in **3·4** and **5·6**). Since exciton coupling is considerably distance dependent ($\propto$ to r^{-2})[52] this indicates a larger separation of the perylenes in hybrid **7·6** than in **3·4** and **5·6**, suggesting an interstrand stacking arrangement of the pyrene and perylene units. Stacking interactions are further supported by UV/Vis absorption spectra (Fig. 3), which show bathochromic shifts for both the pyrene (16 nm) and the perylene (3–4 nm) absorption bands. Concluding, thus, that the pyrene and PDI residues are arranged in an interstrand stacking mode, four possibilities (–**SSEE**–, –**SEES**–, –**SESE**–, –**ESSE**–) exist. Of these, only the alternating, *zipper-like* arrangement of pyrene and PDI units (–**SESE**–) is compatible with the observations (*i.e.* no excimer signal, separation of PDI units and pyrene–PDI stacking interactions).

The efficiency of the quenching process is additionally illustrated in Fig. 4, in which the thermal denaturation of hybrid **7·6** is monitored by the emission at 500 nm. Below 50 °C, the

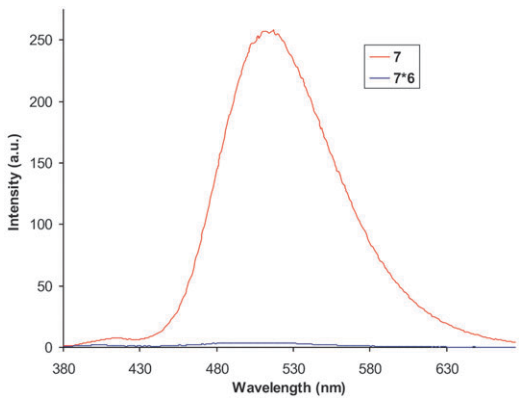


Fig. 1 Fluorescence spectra of single strand **7** and of hybrid **7·6**. Conditions: see Table 1; excitation wavelength: 354 nm.

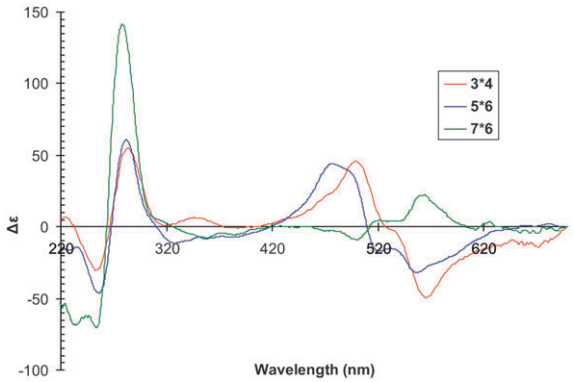


Fig. 2 CD spectra of **3·4**, **5·6** and **7·6** at 30 °C; $\Delta\varepsilon$ (mol^{-1} dm^3 cm^{-1}).

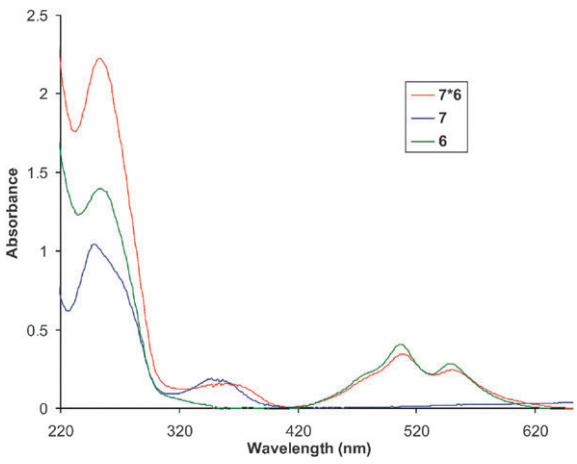


Fig. 3 UV/Vis spectra of hybrid **7·6** and the corresponding single strands. *Conditions*: 2.5 μM oligonucleotide (each strand), 10 mM phosphate buffer (pH 7.4) and 100 mM NaCl.

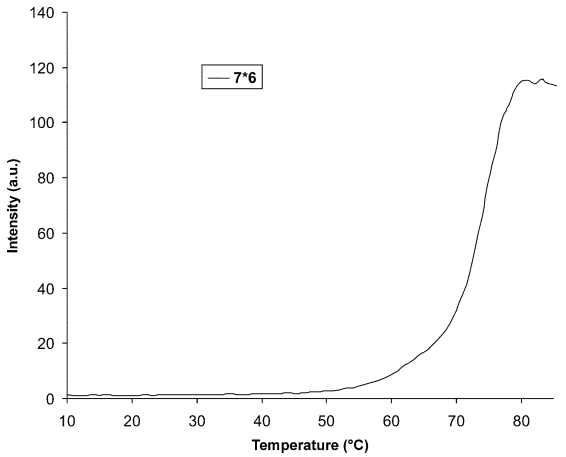


Fig. 4 Temperature-dependent fluorescence of hybrid **7·6**. *Conditions*: see Fig. 3; observed T_m = 74.7 °C.

excimer signal is entirely absent. An increase of the temperature above this value is accompanied by a very sharp transition and maximum fluorescence is obtained after melting of the duplex.

In conclusion, pyrene excimer fluorescence is efficiently quenched by a pair of non-nucleosidic perylene diimide (PDI) building blocks in a DNA duplex. Two factors may account for the quenching effect. First, the excellent spectral overlap between PDI absorbance and pyrene excimer emission should allow an efficient energy transfer. Secondly, pairs of pyrene and PDI units were found to interact *via* interstrand stacking. Thus, the PDI building blocks can physically interfere with the formation of the pyrene excimer leading to a non-fluorescent pyrene-PDI complex. Highly effective excimer quenching is important for many types of molecular probes. The present system may help in the design of diagnostic tools.

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