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Selectivity in DNA interstrand-stacking

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Abstract—The synthesis and spectroscopic investigation of DNA hybrids containing non-nucleosidic pyrene and phenanthrene building blocks is reported. It was found that interstrand-stacking of the polyaromatic compounds in the DNA duplex takes place with a high degree of selectivity.
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Nucleic acids and related kinds of oligomers are of importance as nanometer-sized, functional matter.[1–3] The well-defined arrangement of their building blocks allows the construction of large assemblies in a predictable manner.[4–7] Not surprisingly, the use of nucleic acids as frameworks for the construction of molecular architectures is arising as a focus of interest in nanotechnology.[1,8,9] Furthermore, the combination of nucleotides with non-natural building blocks.[10–18] greatly enhances the variety of possible structures as well as their potential applications. We recently reported the synthesis of non-nucleosidic, polyaromatic building blocks and their incorporation into double stranded DNA.[19] These building blocks serve as surrogates for the natural building blocks without destabilizing the DNA duplex nor altering its overall B-type structure. Spectroscopic investigations support a duplex, in which the polyaromatic residues are arranged in an interstrand-stacked fashion.[20–23] In particular, it was shown that interstrand-stacked pyrenes give rise to excimer formation.[24] Hybridisation-induced excimer formation is well documented.[25–46] Due to the large bathochromic shift of the excimer fluorescence—up to 100 nm compared to the fluorescence of the monomer—such systems are of interest for applications in materials research as well as in genetic diagnostics. The combination of structurally related but electronically disparate non-nucleosidic, polyaromatic building blocks, such as the phenanthrene **P** and the pyrene **S** (see Table 1), opens the possibility of assembling different types of hybrids with diverse spectroscopic properties. Thus, the combination of two single strands, each possessing one phenanthrene and one pyrene building block, may lead to either of the two potential duplex forms illustrated in Scheme 1. Of the possible conformations, only the one having the two pyrenes in adjacent positions (shown on the right) will give rise to an excimer. Thus, the spectroscopic properties of the two types of hybrids will be entirely different and should allow a structural assignment of the formed hybrid. Here, we report the synthesis and structural investigation of DNA hybrids containing unequal non-nucleosidic, polyaromatic building blocks.

The oligomers **1**–**6** used in this study are shown in Table 1. Synthesis and incorporation of the building blocks into the respective oligomers followed the previously described procedures.[20,47] Each of the oligomers **3**–**6** contains a unequal pair of modified building blocks (**S** and **P**) in the middle of the sequence. The control duplex, which is formed by the complementary oligodeoxynucleotides **1** and **2**, has two AT-base pairs in place of the modified building blocks.

To establish the relative arrangement of the phenanthrene and pyrene moieties within the hybrids, the fluorescence spectra of the single and double strands were recorded. As displayed in Figure 1, both hybrids **3*****4** and **5*****6** show a strong band with a maximum intensity at 500 nm, which is characteristic for pyrene excimer emission. The same band is absent in the fluorescence spectra of the single strands. The single strands give rise to weak monomer (<400 nm) as well as exciplex (435 nm) fluorescence. The latter band can be attributed to either the formation of a pyrene–phenanthrene or to a pyrene nucleobase exciplex. Exciplex formation between

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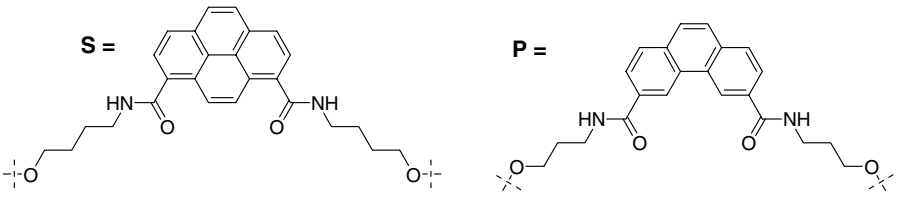
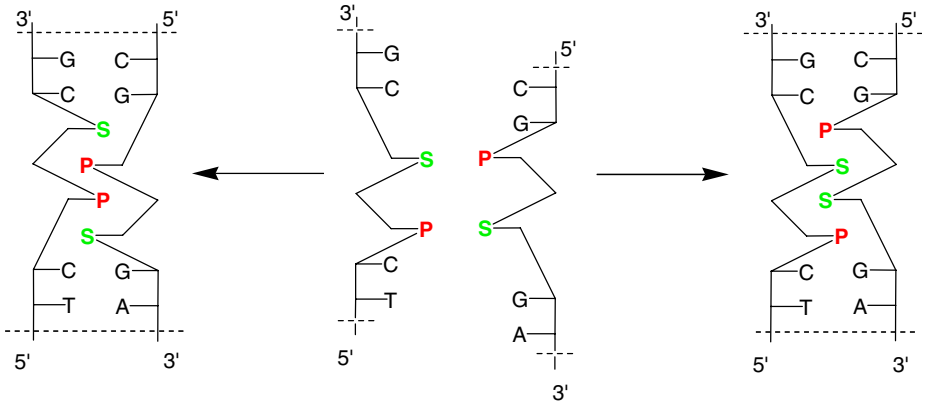
Table 1. Melting temperatures of hybrids containing pyrene and phenanthrene building blocks



Oligomer	Hybrid	T_m (°C)[a]		
		Absorbance 260 nm[b]	Emission 400 nm[b,c]	Emission 500 nm[b,c]
1	(5′) AGC TCG GTC ATC GAG AGT GCA	69.0	—	—
2	(3′) TCG AGC CAG TAG CTC TCA CGT			
3	(5′) AGC TCG GTC **SPC** GAG AGT GCA	68.7	68.0	66.8
4	(3′) TCG AGC CAG **PSG** CTC TCA CGT			
5	(5′) AGC TCG GTC **PSC** GAG AGT GCA	66.9	66.7	66.0
6	(3′) TCG AGC CAG **SPG** CTC TCA CGT			

[a] Conditions: oligomer concentration 1.0 μM, 10 mM Tris–HCl, 100 mM NaCl, pH 7.4; temperature gradient: 0.5 °C/min.
[b] Melting temperatures were determined from the maximum of the first derivative of the melting curve (A_{260} or fluorescence intensity against temperature); experimental error: ±0.5 °C.
[c] Excitation wavelength, 350 nm; excitation slit, 5 nm; emission slit, 10 nm.



Scheme 1. Possible interstrand-stacking arrangements of unequal pairs of phenanthrene (**P**) and pyrene (**S**) building blocks.

pyrene and nucleobases, in particular guanine, has been described.[48,49] However, the exciplex band in single strand **5**—in which guanine is not a nearest neighbour to pyrene—is stronger than in single strand **6**. Therefore, the 435 nm band is most likely due to a pyrene–phenanthrene exciplex. In the spectra of the hybrids, however, bands corresponding to monomer or exciplex fluorescence are almost entirely absent. Of the two possibilities shown in Scheme 1, hybridisation takes place, thus, in the way shown on the right generating the pyrene excimer.[50] A model of the duplex is shown in Figure 2.

The relative duplex stabilities were next assessed in thermal denaturation experiments by monitoring the absorbance at 260 nm. As presented in Table 1, all three hybrids have very similar melting temperatures (T_m). The duplex **3*****4** has a T_m of 68.7 °C, almost identical to the one of the parent duplex (69 °C). The T_m of the hybrid **5*****6** is approximately 2 °C lower. Thus, the non-nucleosidic phenanthrene and pyrene building blocks have no or only a small negative effect on the structural stability of the duplex. This is in agreement with our previous findings, in which non-nucleosidic building blocks of this type were shown to be well tolerated in a B-type duplex.[20,52]

The formation of the pyrene excimer upon annealing of the strands allows the observation of the hybridisation process also by monitoring the respective fluorescence signals. The thermal denaturation curves obtained with hybrid **5*****6** are displayed in Figure 3. Fluorescence was measured at 500 nm (excimer) and at 400 nm (monomer). While, as expected, the signal intensity of the excimer decreases with increasing temperature, the monomer fluorescence increases simultaneously. The T_m values derived from the curves are in excellent agreement with the ones obtained from the absorbance at 260 nm, differing by less than 2 °C (see Table 1). This demonstrates that the pyrene excimer formation is a temperature- and thus hybridisation-dependent process thereby confirming that the interstrand-stacking interaction of the modified building blocks is an integral part of the hybridisation process.

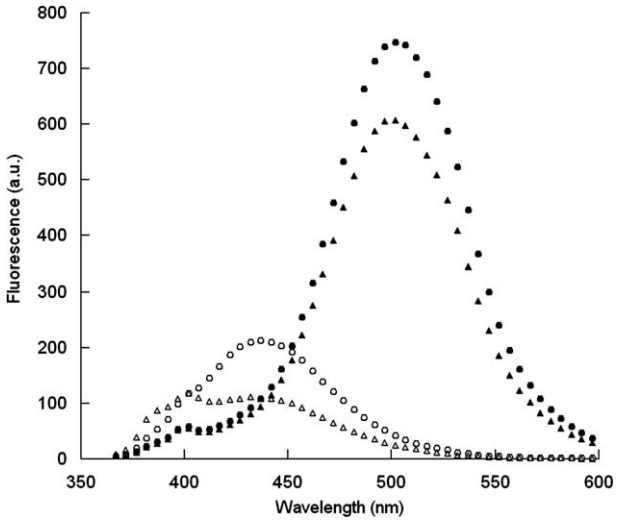


Figure 1. Fluorescence spectra of pyrene- and phenanthrene-containing oligonucleotides: duplex **3**∗**4** (▲); duplex **5**∗**6** (●); single strands **5** (○) and **6** (△); spectra of the single strands **3** and **4** are omitted for clarity. Conditions: 1.0 mM oligomer concentration, 10 mM Tris–HCl, 100 mM NaCl, pH 7.4, room temperature. Excitation wavelength: 354 nm; excitation slit: 5 nm; emission slit: 7 nm.

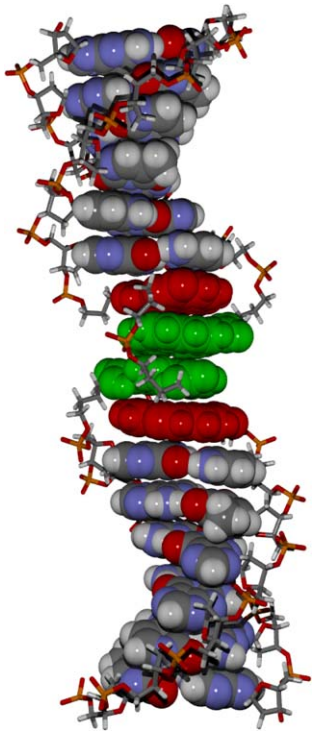


Figure 2. Amber-minimized[51] model duplex **5**∗**6** giving rise to formation of a pyrene excimer. Pyrene building blocks are shown in green, phenanthrenes in red.

The data described above show that non-nucleosidic pyrene and phenanthrene building blocks interact in a highly selective way. The interaction between the polyaromatic compounds, which involves interstrand-stacking, leads to pyrene–pyrene and not to phenanthrene–phenanthrene contacts. The spectroscopic properties of the formed hybrids are largely controlled by the relative

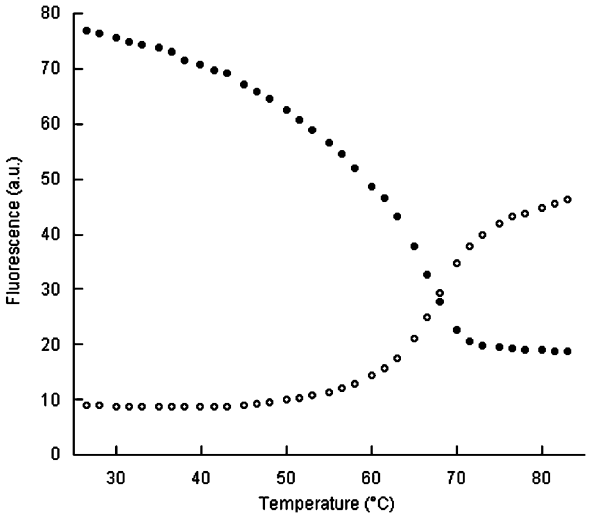


Figure 3. Thermal denaturation curves of duplex **5**∗**6** recorded by monitoring the excimer (500 nm; filled circles) and monomer (400 nm; open circles) fluorescence. Excitation wavelength: 354 nm; excitation slit: 5 nm; emission slit: 7 nm. Oligomer concentration 1.0 μM, 10 mM Tris–HCl, 100 mM NaCl, pH 7.4; temperature gradient: 0.5 °C/min.

arrangement of the modified building blocks. Oligonucleotides containing such building blocks may, thus, find applications in the construction of fluorescent nanomaterials or as probes for diagnostic purposes.

In conclusion, we have synthesised and investigated DNA mimics containing unequal pairs of non-nucleosidic, interstrand-stacking phenanthrene and pyrene building blocks. Spectroscopic investigation of the hybrids formed by the modified oligomers reveals that the interstrand-stacking interactions are taking place in a selective way, leading to formation of pyrene–pyrene rather than phenanthrene–phenanthrene interactions. The findings are important for the design of DNA mimics composed of interstrand-stacking building blocks possessing interesting spectroscopic and physicochemical properties.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.07.039.

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50. It is worthy to mention that in both double strands (**3*****4** and **5*****6**) no exciplex band or shoulder can be observed. This allows largely ruling out the formation of the alternative structure (left side of Scheme 1) since in such hybrid significant exciplex fluorescence would have to be observed.
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