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1-Ethynylpyrene-modified guanine and cytosine as optical labels for DNA hybridization†

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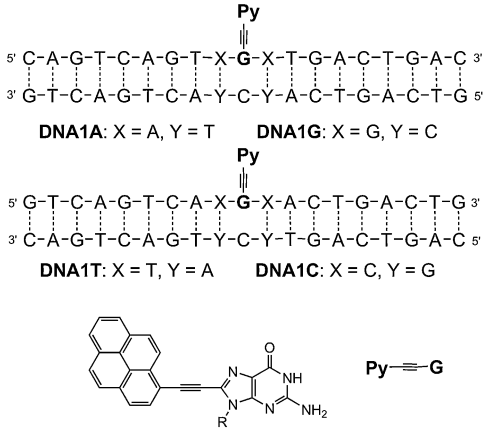
1-Ethynylpyrene shows remarkable absorption changes upon DNA hybridization when it is covalently attached to the 8-position of guanine. An absorption band at ~420 nm is only present in the duplex, exhibits thermal melting behaviour and provides the basis for a molecular beacon together with 1-ethynylpyrene-modified cytosine.

Various analytical problems in biotechnology and biomedicine require suitable labels for optical spectroscopy methods with DNA, *e.g.* fluorescence resonance energy transfer.[1] Various organic dyes have been attached covalently to oligonucleotides and applied as probes for DNA hybridization and as molecular beacons.[2] For instance, thiazole orange exhibits a significant fluorescence which is sensitive to the environment in DNA–PNA duplexes and thus can be applied for the detection of base mismatches.[3] Additionally, there is an increasing demand for DNA probes with duplex-sensitive absorption properties.[4] Recently, we[5] and others[6] introduced the 1-ethynylpyrene moiety as a versatile and tunable fluorescent probe for DNA. The inflexible acetylene linker is able to couple chromophores electronically to the nucleobases in order to affect their emission properties in potentially useful ways.[7] Herein, we want to describe detailed studies of the optical properties of 8-(1-ethynylpyrene)-modified guanine (Py–≡–dG) in duplex DNA and its application in fluorescence resonance energy transfer experiments together with the chemically very similar 5-(1-ethynylpyrene)-cytosine (Py–≡–dC) modification.

The Py–≡–dG- and Py–≡–dC-modified oligonucleotides were synthesized by a semi-automated synthetic strategy using Sonogashira-type cross-couplings on solid phase.[5,8] Using this protocol, a time-consuming synthesis of the corresponding phosphoramidites[6] can be avoided since the procedure is based on commercially available DNA building blocks.

We prepared first a set of Py–≡–dG-modified DNA duplexes (**DNA1X**, Scheme 1) that differ in the base pairs (X–Y) adjacent to the modification site. The UV/Vis spectra of all hybridized duplexes **DNA1X** show a significant absorption band at ~421 nm. Representatively, Fig. 1 shows the temperature-dependent UV/Vis spectra of **DNA1C**. At temperatures above 40 °C, the absorption band at ~421 nm collapses nearly completely. Moreover, the temperature-dependent UV/Vis spectrum of **DNA1C** shows an isosbestic point at ~380 nm indicating a single cooperative transition from the Py–≡–dG-modified duplex to the corresponding single strand. Interestingly, the maximum of the absorbance band at ~421 nm does not depend on the neighboring base pairs X–Y excluding an exciplex-type excited state with charge-separated character. Thus, this absorption band can be attributed to a pure excitonic interaction between the Py–≡–dG-unit and the adjacent base pairs which occurs only upon hybridization and thus requires the DNA duplex conformation.



Scheme 1 Py–≡–dG-modified DNA duplex set **DNA1X**.

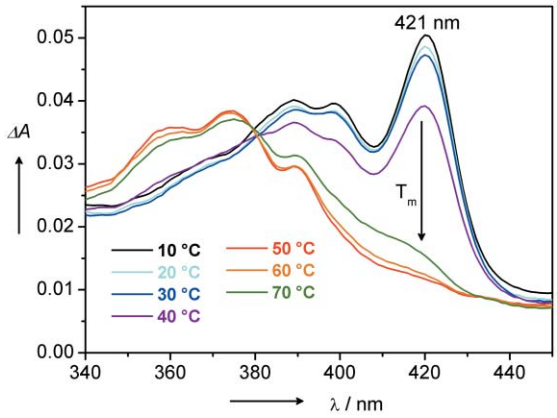


Fig. 1 Temperature-dependent UV/Vis absorption spectra of **DNA1C** (2.5 μM in 10 mM Na–P_i buffer, pH 7).

The temperature-dependent absorbance changes of the Py–≡–dG-modified duplexes were recorded at 260 nm (DNA melting temperatures, T_m) and additionally at 421 nm (Table 1). Since the absorption band at 421 nm is the result of a ground-state interaction between the Py–≡–dG-chromophore and the adjacent base pairs, the temperature-dependent destacking at 421 nm is highly sequence selective and occurs about 2–15 °C lower than the melting temperatures of the DNA duplexes.

Table 1 Melting temperatures (T_m) at 260 nm and absorbance changes (ΔA) at 421 nm of the Py–≡–dG-modified duplexes **DNA1X** (1.25 μM) in Na–P_i buffer (10 mM, 250 mM NaCl, pH 7)

Duplex	T_m (260 nm)	ΔA (421 nm)
DNA1A	68 ± 1 °C	53 ± 2 °C
DNA1G	65 ± 1 °C	63 ± 2 °C
DNA1T	68 ± 1 °C	52 ± 2 °C
DNA1C	66 ± 1 °C	55 ± 2 °C

† Electronic supplementary information (ESI) available: Experimental details, Scheme S1, Table S1 and Figures S1–S10. See http://www.rsc.org/suppdata/ob/b5/b504079e/

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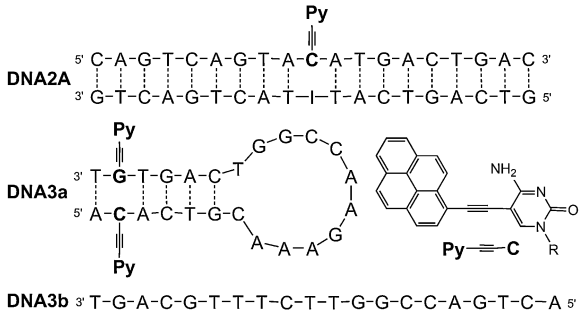
In further experiments we applied this characteristic duplex absorption of Py–≡–dG-modified DNA in fluorescence energy transfer experiments. The Foerster-type of energy transfer requires a significant overlay of the emission of the energy donor with the absorption of the acceptor. Based on our previous results[5] the emission of Py–≡–dC-modified DNA fits this requirement. We show representatively **DNA2A** (Scheme 2) and compared the optical properties with that of **DNA1T** showing a significant overlay of the emission of Py–≡–dC with the absorption of Py–≡–dG between 400–430 nm (Fig. 2). The absorption and emission maxima do not depend on the sequential context of the adjacent DNA bases and hence switching of the energy donor and acceptor properties of Py–≡–dC and Py–≡–dG, respectively, can not occur in different sequences.



Scheme 2 Sequences of **DNA2A, DNA3a** and **DNA3b**.

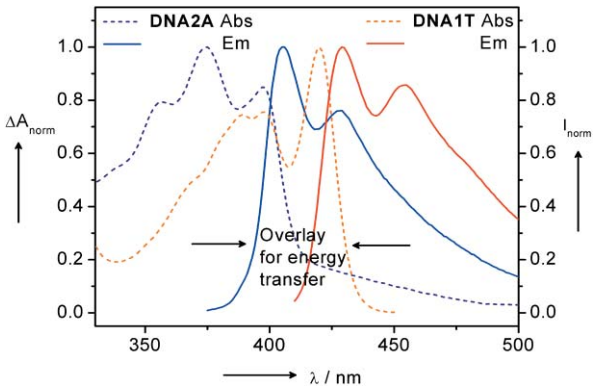


Fig. 2 Normalized UV/Vis absorption and fluorescence spectra of **DNA1T** (λ_{exc} 391 nm) and **DNA2A** (λ_{exc} 360 nm) (2.5 μM) in 10 mM Na–P_i buffer, pH 7.

The application of Py–≡–dC as a second label which is chemically very similar to Py–≡–dG has the advantage that both modifications can be introduced by the same solid-phase methodology using commercially available reagents in a single coupling mixture. Accordingly, we prepared the double labeled hairpin **DNA3a**. As expected, an energy transfer can be obtained in **DNA3a** (Fig. 3) since it shows the typical emission of Py–≡–dG although it has been excited at 350 nm where the Py–≡–dC chromophore exhibits a substantially higher extinction than Py–≡–dG. Interestingly, the fluorescence of **DNA3a** changes remarkably when it is hybridized with the complementary oligonucleotide **DNA3b**. The resulting duplex **DNA3a**–**3b** shows mainly the typical emission of Py–≡–dC indicating that the

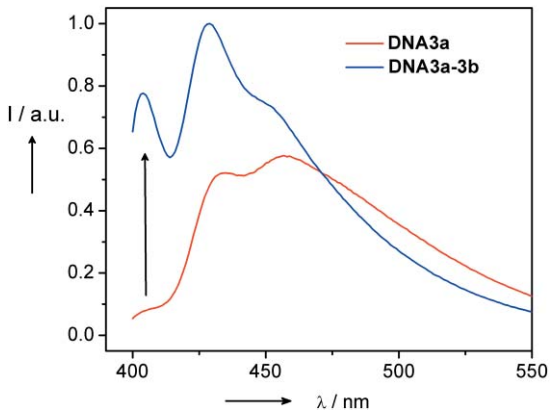


Fig. 3 Fluorescence spectra of hairpin **DNA3a** and duplex **DNA3a-3b** (λ_{exc} 350 nm, 2.0 μM in 10 mM Na–P_i buffer, pH 7).

energy transfer to Py–≡–dG has been blocked due to the large distance in the duplex.

In conclusion, we showed that 1-ethynylpyrene is a versatile optical label which can be introduced twice by a single solid-phase methodology. More importantly, the kind of nucleobase to which the 1-ethynylpyrene is attached decides between energy donor (Py–≡–dC) and acceptor (Py–≡–dG) properties. Hence, this label can be incorporated wherever it is needed in the genetic context. This indicates a high potential of this type of assay in DNA analytics.

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References

1 For reviews see *e.g.*: S. Tyagi, S. A. E. Marras and F. R. Kramer, *Nature Biotechnol.*, 2000, **18**, 1191; D. M. J. Lilley and T. J. Wilson, *Curr. Opin. Chem. Biol.*, 2000, **4**, 507; M. E. Hawkins, *Cell. Biochem. Biophys.*, 2001, **34**, 257; T. Heyduk and E. Heyduk, *Nature Biotechnol.*, 2002, **20**, 171.

2 For reviews see *e.g.*: M. K. Johansson and R. M. Cook, *Chem. Eur. J.*, 2003, **9**, 3466; W. Tan, K. Wang and T. J. Drake, *Curr. Opin. Chem. Biol.*, 2004, **8**, 547.

3 O. Köhler, D. V. Jarikote and O. Seitz, *ChemBioChem*, 2005, **6**, 69.

4 H. Asanuma, H. Kashida, X. Liang and M. Komiyama, *Chem. Commun.*, 2003, 1536.

5 M. Rist, N. Amann and H.-A. Wagenknecht, *Eur. J. Org. Chem.*, 2003, 2498; E. Mayer, L. Valis, C. Wagner, M. Rist, N. Amann and H.-A. Wagenknecht, *ChemBioChem*, 2004, **5**, 865.

6 A. D. Malakhov, E. V. Malakhova, S. V. Kuznitsova, I. V. Grechishnikova, I. A. Prokhorenko, M. V. Skorobogatyi, V. A. Korshun and Y. A. Berlin, *Russ. J. Bioorg. Chem.*, 2000, **26**, 39; G. T. Hwang, Y. J. Seo, S. J. Kim and B. H. Kim, *Tetrahedron Lett.*, 2004, **45**, 3543; G. T. Hwang, Y. J. Seo and B. H. Kim, *Tetrahedron Lett.*, 2005, **46**, 1475; A. Okamoto, Y. Ochi and I. Saito, *Chem. Commun.*, 2005, 1128.

7 D. Hurley and Y. Tor, *J. Am. Chem. Soc.*, 2002, **124**, 3749; L. H. Thoresen, G.-S. Jiao, W. C. Haaland, M. L. Metzker and K. Burgess, *Chem. Eur. J.*, 2003, **9**, 4603; V. L. Andronova, M. V. Skorobogatyi, E. A. Manasova, Y. A. Berlin, V. A. Korshun and G. A. Galegov, *Russ. J. Bioorg. Chem.*, 2003, **29**, 262; A. D. Malakhov, M. V. Skorobogatyi, I. A. Prokhorenko, S. V. Gontarev, D. T. Kozhich, D. A. Stetsenko, I. A. Stepanova, Z. O. Shenkarev, Y. A. Berlin and V. A. Korshun, *Eur. J. Org. Chem.*, 2004, 1298; G. T. Hwang, Y. J. Seo and B. H. Kim, *J. Am. Chem. Soc.*, 2004, **126**, 6528; T. Kottysch, C. Ahlborn, F. Brotzel and C. Richert, *Chem. Eur. J.*, 2004, **10**, 4017.

8 S. I. Khan and M. W. Grinstaff, *J. Am. Chem. Soc.*, 1999, **121**, 4704.