1-Ethynylpyrene-Modified Guanine and Cytosine as Optical Labels for DNA Hybridization

Supporting Information

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Materials and Methods

Solvents were dried according to standard procedures. The Sonogashira couplings were carried out under argon. Chemicals were purchased from Sigma-Aldrich or Lancaster and used without further purification. MALDI-TOF was performed in the analytical facility of the department on a Bruker Biflex III spectrometer using 3-hydroxypicolinic acid in aq. ammonium citrate as the matrix. C18-RP analytical and semipreparative HPLC columns (300 Å) were purchased from Supelco. All spectroscopic measurements were performed in quartz glass cuvettes (1 cm) from Starna Cells. Absorption spectra were recorded on a Varian Cary 100 or on a Varian Cary 50 spectrometer. Melting curves were measured with DNA duplex (1.25 μM) in Na-P_i buffer solution (10 mM) with NaCl (250 mM), pH 7.0. The temperature was controlled by a Cary temperature control unit, and was increased from 10 to 80 °C at a heating rate of 0.5 °C/min. The fluorescence spectra were recorded on a Fluoromax-3 fluorimeter (Jobin-Yvon). All emission spectra were recorded with a bandpass of 2 nm for both excitation and emission and are intensity corrected.

Preparation of Oligonucleotides and DNA Duplexes (General Procedure). The unmodified oligonucleotides were prepared on an Expedite 8909 DNA synthesizer from Applied Biosystems via standard phosphoramidite chemistry. Phosphoramidites and CPGs (1 μmol) were purchased from Glen Research or ABI, all other synthesizer chemicals only from ABI. After preparation, the trityl-off oligonucleotide was cleaved off the resin and was deprotected by treatment with conc. NH_4OH at 60 °C for 10 h. The oligonucleotide was dried and purified by HPLC on a semipreparative RP-C18 column (300 Å, Supelco) using the following conditions: A=NH_4OAc buffer (50 mM), pH=6.5; B=MeCN; gradient=0-15 % B over 45 min. The oligonucleotides were lyophilized and quantified by their absorbance at 260 nm (see: J. D. Puglisi, I. Tinoco, *Meth. Enzymol.*, 1989, **180**, 304.). Duplexes were formed by heating to 80 °C (10 min.) followed by slow cooling.

Preparation of Py-≡-dG and Py-≡-dC-modified oligonucleotides . The solid-phase synthesis sequence was stopped after the incorporation of 5'-DMT-3'-cyanoethyl-*N*,*N*'-diisopropyl phosphoramidite-2'-deoxy-8-bromo-guanosine or 5'-DMT-3'-cyanoethyl-*N*,*N*'-diisopropyl phosphoramidite-2'-deoxy-5-iodocytidine (Glen Research) without deprotecting the 5'-hydroxyl or cleaving the oligonucleotide from the resin. In case of the double labeled oligonucleotide **DNA3a** the coupling procedure was performed at each modification site. The column was subsequently removed from the synthesizer and dried in vacuo. Then the column was attached to a syringe and the reaction solution consisting of ethynylpyrene (60 µmol), $Pd(Ph_3P)_4$ (30 µmol) and CuI (30 µmol) in dry DMF:Et_3N (3.5:1.5) (0.5 mL) was prepared under argon and injected into the column and into another syringe, attached to the other end of the column. The reaction solution was moved back and forth between the two syringes through the column several times to ensure even distribution of the reaction solution. After a coupling time of three hours at r. t., the reaction solution was discarded, the column washed with DMF:Et_3N (9:1) (10 mL) and dry MeCN (40 mL), dried in vacuo, and reinstalled on the synthesizer. Solid-phase synthesis was resumed, and additional DNA bases were added. The deprotection, cleavage from resin and purification by HPLC was performed as described above, but the gradient for HPLC purification was run from 0-30 % MeCN over 45 min. The concentrations of the oligodeoxynucleotides containing Py-≡-dC or Py-≡-dG were determined following the absorption at 260 nm as described previously (see M. Rist, N. Amann, H.-A. Wagenknecht, *Eur. J. Org. Chem.* **2003**, 2498; and E. Mayer, L. Valis, C. Wagner, M. Rist,, N. Amann, H.-A. Wagenknecht, *ChemBioChem,* **2004**, *5*, 865). Masses of modified oligonucleotides were determined by MALDI-TOF mass spectrometry to confirm the correct base sequence and the successful incorporation of the ethynylpyrene group (Table S1). Duplexes with 1.2 eq. of the unmodified complementary strand were formed by heating to 80 °C (10 min.), followed by slow cooling to r.t. over 4 h.

Scheme S1: Py-≡-dC-modified DNA duplex set **DNA2X** (I=inosine)**.**

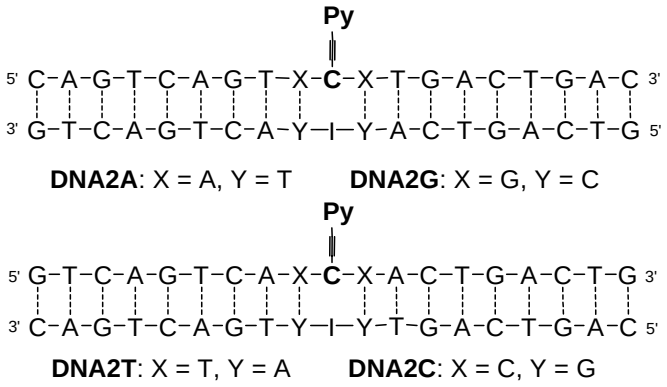

Table S1

Calculated and experimentally determined masses of the modified single stranded oligonucleotides of **DNA1X**(ss), **DNA2X**(ss) and **DNA3a**.

oligonucleotide	mass calc.	mass exp.
DNA1A(ss)	6061	6066
DNA1G(ss)	6093	6095
DNA1T(ss)	6043	6047
DNA1C(ss)	6013	6017
DNA2A(ss)	5973	5973
DNA2G(ss)	5773	5780
DNA2T(ss)	5821	5824
DNA2C(ss)	5853	5856
DNA3a	7820	7827

Figure S1

Temperature-dependent UV/Vis absorption spectra of **DNA1A** (2.5 µM in 10 mM Na-P_i buffer, pH 7).

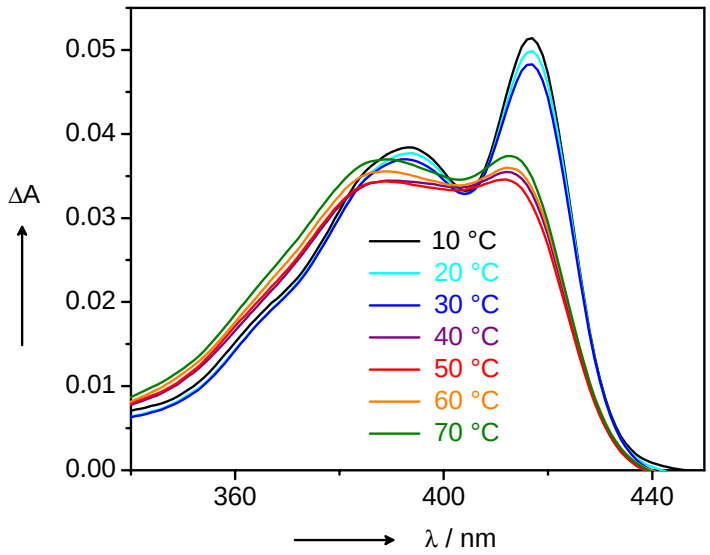

Figure S2

Temperature-dependent UV/Vis absorption spectra of **DNA1G** (2.5 µM in 10 mM Na-P_i buffer, pH 7).

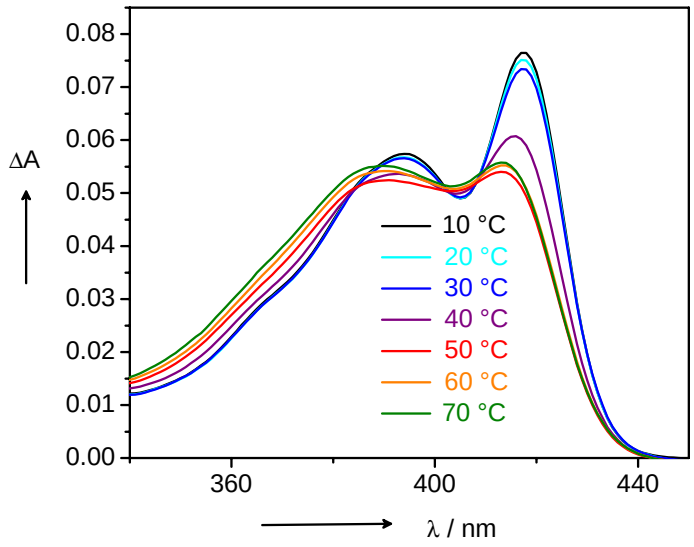

Figure S3

Temperature-dependent UV/Vis absorption spectra of **DNA1T** (2.5 µM in 10 mM Na-P_i buffer, pH 7).

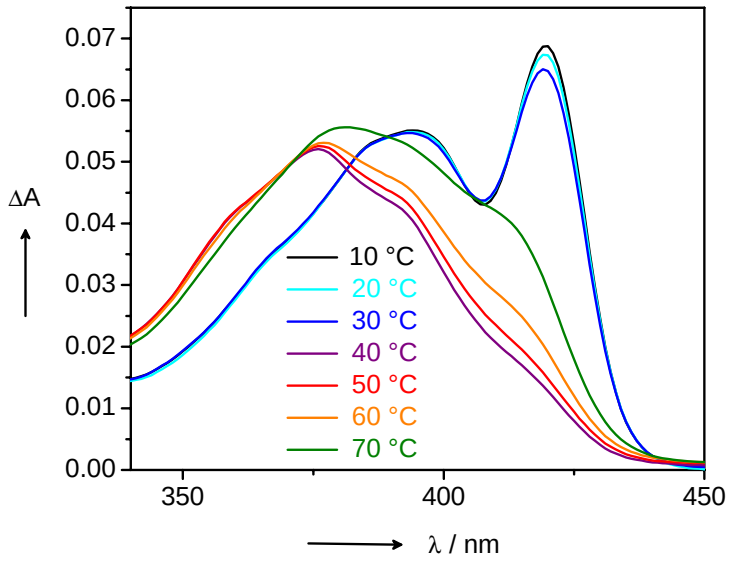

Figure S4

Fluoresence spectra of **DNA1X** (2.5 µM in 10 mM Na-P_i buffer, pH 7, λ_{exc} 391 nm).

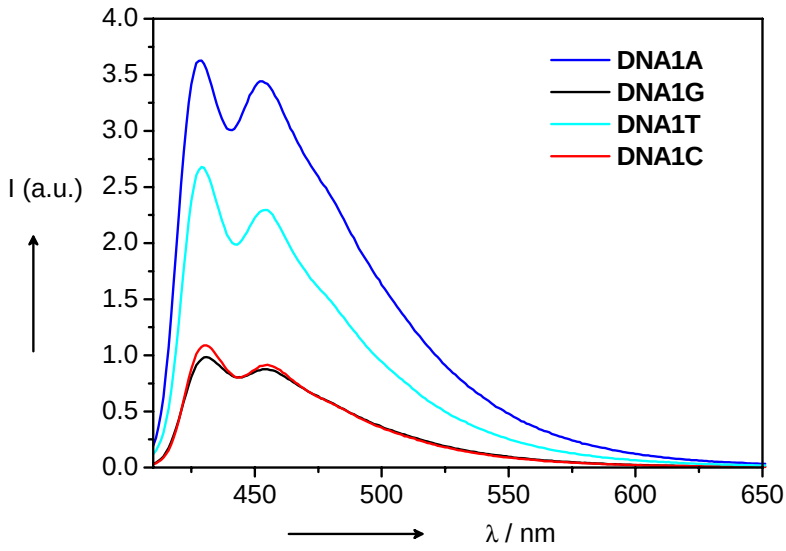

Figure S5

UV/Vis absorption spectra of **DNA2X** (2.5 µM in 10 mM Na-P_i buffer, pH 7).

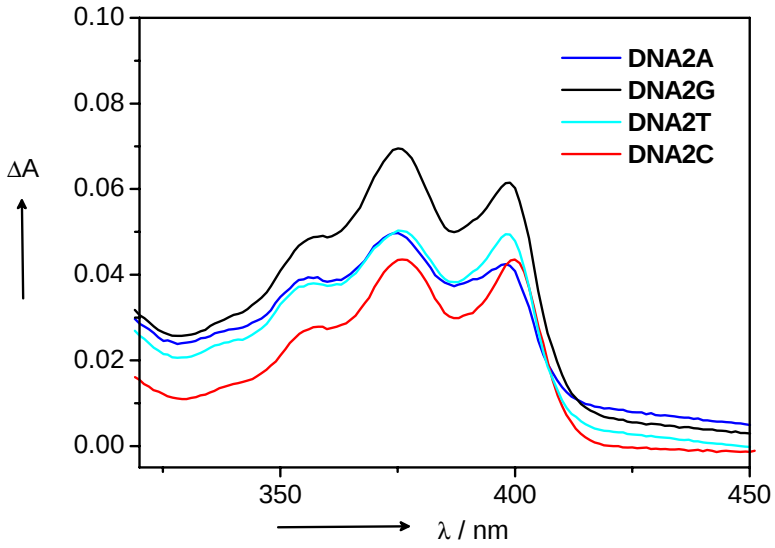

Figure S6

Fluoresence spectra of **DNA2X** (2.5 µM in 10 mM Na-P_i buffer, pH 7, λ_{exc} 360 nm).

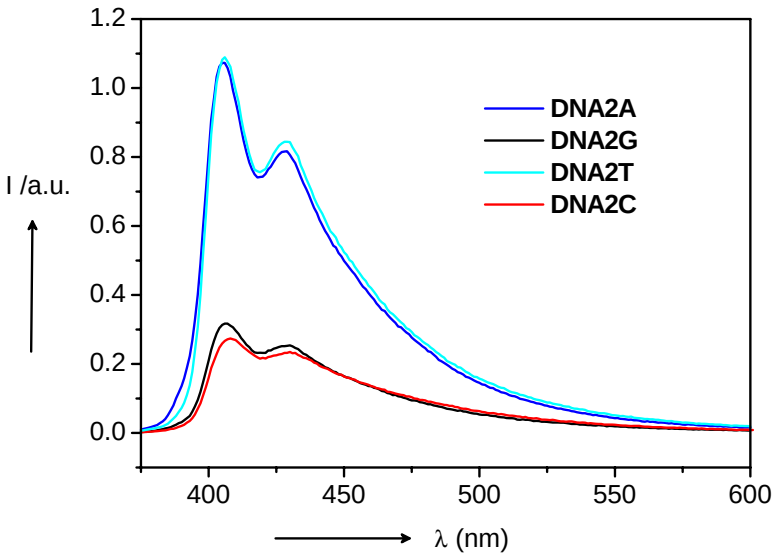

Figure S7

Melting curves of **DNA2X** (1.25 μM in 10 mM Na-P_i buffer, 250 mM NaCl, pH 7), λ 260 nm.

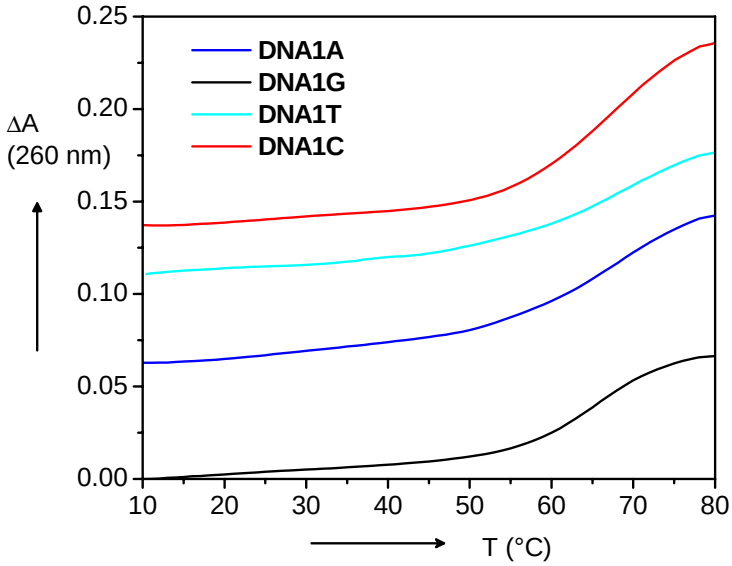

Figure S8

Temperature dependent absorption changes of **DNA1X** (1.25 μM in Na-P_i 10 mM buffer, 250 mM NaCl, pH 7), λ 421 nm .

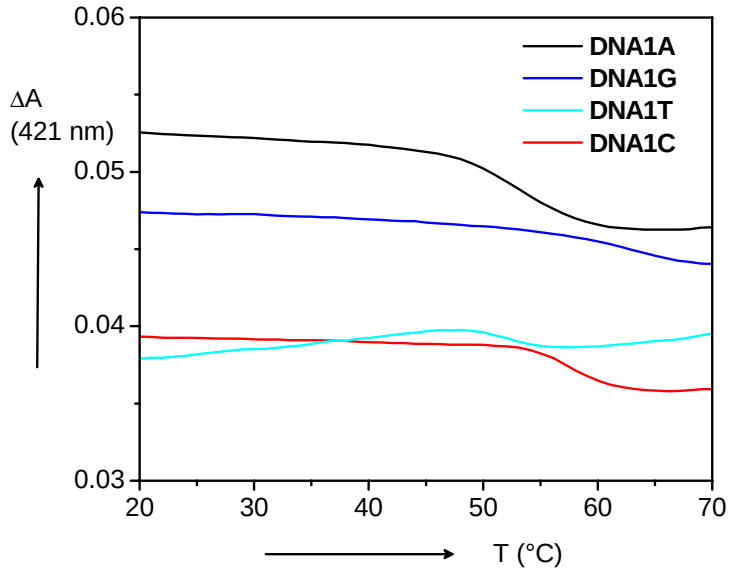

Figure S9

UV/Vis absorption spectra of single-stranded oligonucleotides **DNA1X**(ss) (2.5 µM in 10 mM Na-P_i buffer, pH 7).

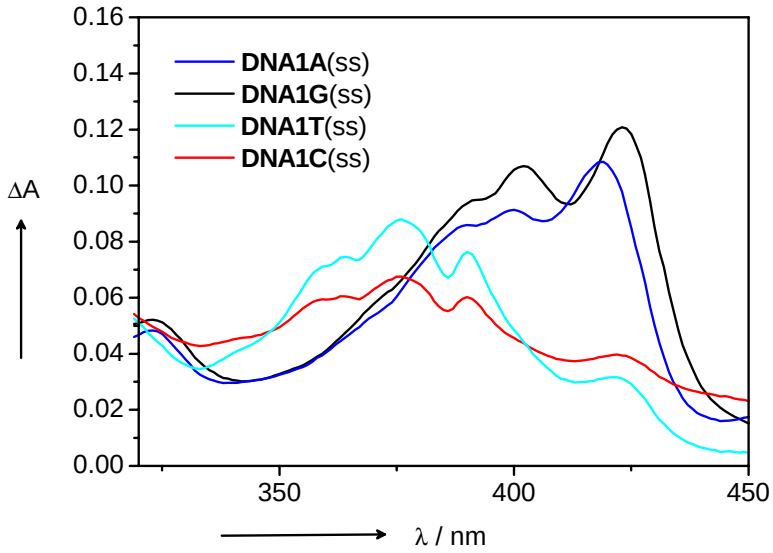

Figure S10

UV/Vis absorption spectra of hairpin **DNA3a** and duplex **DNA3a-3b** (2.0 µM in 10 mM Na-P_i buffer, pH 7).