Supporting Information

Detection of Single Base Mismatches and Abasic Sites Using Phenanthridinium as an Artificial DNA Base and Charge Donor

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Materials and methods. 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 and C5-RP analytical and semipreparative HPLC columns (300 Å) were purchased from Supelco. All spectroscopic measurements were performed in quartz glass cuvettes (1 cm, pump-probe laser spectroscopy: 1 mm) and using Na-P_i-buffer (10 mM). The melting temperatures (2.5 μM duplex, 250 mM NaCl, 260 nm, 10-90 °C, interval 0.5-1 °C) were recorded on a Varian Cary Bio 100 spectrometer. The absorption spectra (12.5 μM duplex) were recorded on a Varian Cary 100 spectrometer and fluorescence spectra (12.5 μM duplex) on a Fluoromax-3 fluorimeter (Jobin-Yvon) and corrected for Raman emission from the buffer solution. All emission spectra were recorded with a bandpass of 2 nm for both excitation and emission and are intensity corrected.

Preparation and characterization of the oligonucleotides (general procedure). The oligonucleotides were prepared on a Expedite 8909 DNA synthesizer from Applied Biosystems via standard phosphoramidite protocols using chemicals and CPG (1 μmol) 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**, 304-325.) on a Varian Cary Bio 100 spectrometer. Duplexes were formed by heating of the phenanthridinium-modified oligonucleotides in the presence of 1.2 equiv. unmodified complementary strand to 90 °C (10 min.), followed by slow cooling.

Preparation of phenanthridinium-modified oligonucleotides. The syntheses were performed on a 1 μmol scale (CPG 500 Å, Glen Research) using standard phosphoramidite protocols. Nearly quantitative coupling of the phenanthridinium building block (see: R. Huber, N. Amann, H.-A. Wagenknecht, *J. Org. Chem.*, 2004, **69**, 744-751.) was achieved using an extended coupling time (1 h instead of 1.5 min. for standard couplings), a higher phosphoramidite concentration (0.2 M instead of 0.067 M), and three coupling cycles interrupted by intermediate washing steps. After preparation, the trityl-off oligonucleotide was cleaved off the resin and was deprotected by treatment with conc. NH_4OH at 60 °C for 5 h. The oligonucleotide was dried and purified by HPLC on a semipreparative RP-C5 wide pore 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, quantified by their absorbance at 260 nm (see: J. D. Puglisi, I. Tinoco, *Meth. Enzymol.*, 1989, **180**, 304-325.) and using ε(260 nm)=45.200 $M^{-1}cm^{-1}$ for E (J. Pauluhn, A. Naujok, H. W. Zimmermann, *Z. Naturforsch.*, 1980, **35**, 585-598.).

Table S1. MS (MALDI-TOF) data of the E-modified oligonucleotides **ssDNA1-X** and **ssDNA2-X**.

oligonucleotide	m/z (calcd.)	m/z(exp.)
DNA1-A	5068	5072
DNA1-C	5044	5048
DNA1-G	5084	5093
DNA1-T	5059	5073
DNA2-A	5069	5073
DNA2-C	5045	5048
DNA2-G	5085	5088
DNA2-T	5060	5062

Table S2. Melting temperatures (T_m) of duplexes **DNA1-XY** and **DNA2-XY**.

DNA	T_m	DNA	T_m	DNA	T_m	DNA	T_m
DNA1-AA	76 °C	**DNA1-GA**	75 °C	**DNA2-AA**	76 °C	**DNA2-GA**	70 °C
DNA1-AC	75 °C	**DNA1-GC**	71 °C	**DNA2-AC**	75 °C	**DNA2-GC**	74 °C
DNA1-AG	77 °C	**DNA1-GG**	74 °C	**DNA2-AG**	73 °C	**DNA2-GG**	72 °C
DNA1-AT	71 °C	**DNA1-GT**	75 °C	**DNA2-AT**	77 °C	**DNA2-GT**	70 °C
DNA1-AS	77 °C	**DNA1-GS**	74 °C	**DNA2-AS**	75 °C	**DNA2-GS**	71 °C
DNA1-CA	75 °C	**DNA1-TA**	78 °C	**DNA2-CA**	74 °C	**DNA2-TA**	76 °C
DNA1-CC	77 °C	**DNA1-TC**	77 °C	**DNA2-CC**	74 °C	**DNA2-TC**	73 °C
DNA1-CG	70 °C	**DNA1-TG**	76 °C	**DNA2-CG**	70 °C	**DNA2-TG**	74 °C
DNA1-CT	75 °C	**DNA1-TT**	77 °C	**DNA2-CT**	75 °C	**DNA2-TT**	76 °C
DNA1-CS	77 °C	**DNA1-TS**	78 °C	**DNA2-CS**	77 °C	**DNA2-TS**	75 °C

Figure S1. Fluorescence spectra of duplexes **DNA1-AY** and **DNA2-AY** (12.5 μM), Na-P_i-buffer (10 mM), pH=7, 25 °C, excitation at 530 nm.

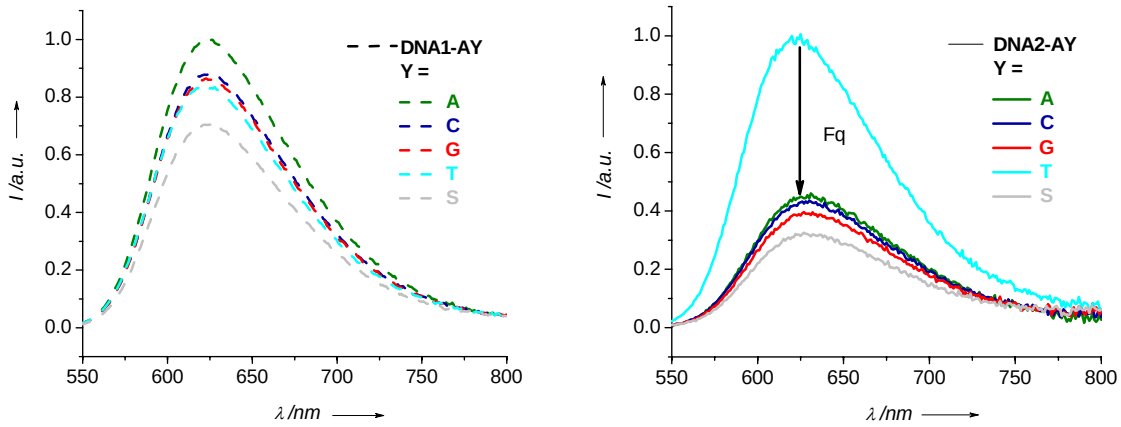

Figure S2. Fluorescence spectra of duplexes **DNA1-GY** and **DNA2-GY** (12.5 μM), Na-P_i-buffer (10 mM), pH=7, 25 °C, excitation at 530 nm.

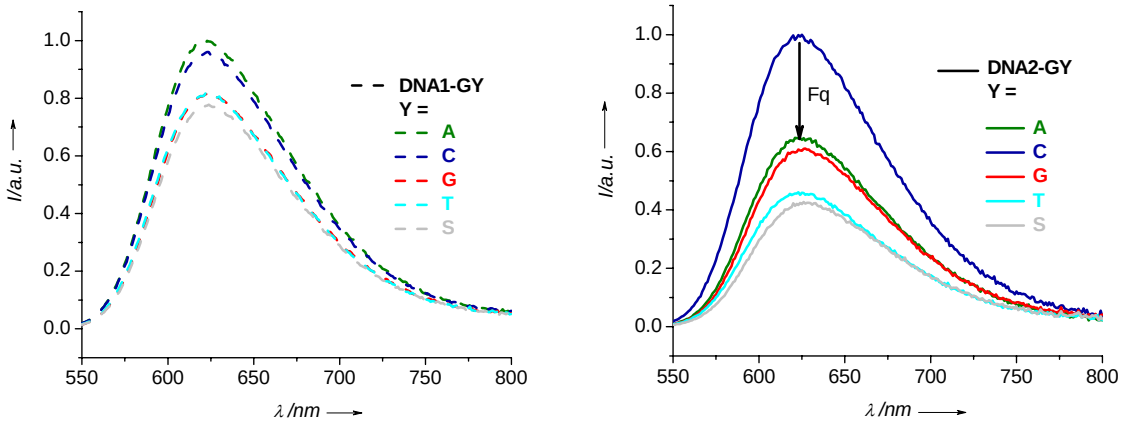

Figure S3. Fluorescence spectra of duplexes **DNA1-TY** and **DNA2-TY** (12.5 μM), Na-P_i-buffer (10 mM), pH=7, 25 °C, excitation at 530 nm.

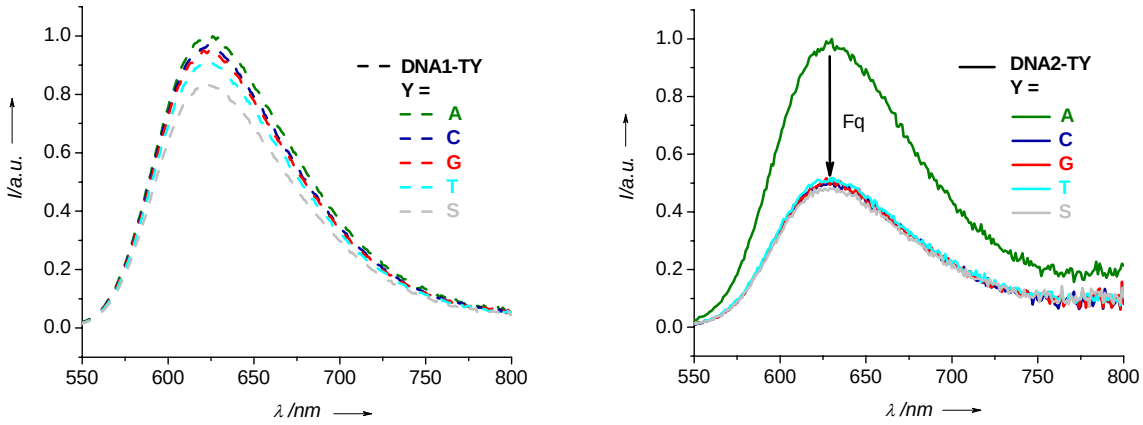

Table S3. MS (MALDI-TOF) data of the phenanthridinium-modified oligonucleotides **ssDNA3-X** and **ssDNA4-X**.

oligonucleotide	m/z (calcd.)	m/z(exp.)
DNA3-A	5068	5078
DNA3-C	5044	5069
DNA3-G	5084	5104
DNA3-T	5059	5066
DNA4-A	5069	5076
DNA4-C	5045	5049
DNA4-G	5085	5088
DNA4-T	5060	5063

Table S4. Melting temperatures (T_m) of duplexes **DNA3-XY** and **DNA4-XY**.

DNA	T_m	DNA	T_m	DNA	T_m	DNA	T_m
DNA3-AA	71 °C	**DNA3-GA**	72 °C	**DNA4-AA**	72 °C	**DNA4-GA**	76 °C
DNA3-AC	74 °C	**DNA3-GC**	71 °C	**DNA4-AC**	72 °C	**DNA4-GC**	72 °C
DNA3-AG	76 °C	**DNA3-GG**	76 °C	**DNA4-AG**	77 °C	**DNA4-GG**	77 °C
DNA3-AT	74 °C	**DNA3-GT**	71 °C	**DNA4-AT**	74 °C	**DNA4-GT**	75 °C
DNA3-AS	71 °C	**DNA3-GS**	75 °C	**DNA4-AS**	71 °C	**DNA4-GS**	72 °C
DNA3-CA	72 °C	**DNA3-TA**	74 °C	**DNA4-CA**	72 °C	**DNA4-TA**	76 °C
DNA3-CC	72 °C	**DNA3-TC**	72 °C	**DNA4-CC**	71 °C	**DNA4-TC**	75 °C
DNA3-CG	74 °C	**DNA3-TG**	76 °C	**DNA4-CG**	72°C	**DNA4-TG**	79 °C
DNA3-CT	73 °C	**DNA3-TT**	72 °C	**DNA4-CT**	72 °C	**DNA4-TT**	75 °C
DNA3-CS	74 °C	**DNA3-TS**	73 °C	**DNA4-CS**	73 °C	**DNA4-TS**	76 °C

Figure S4. Fluorescence spectra of duplexes **DNA3-AY** and **DNA4-AY** (12.5 µM), Na-P_i-buffer (10 mM), pH=7, 25 °C, excitation at 530 nm.

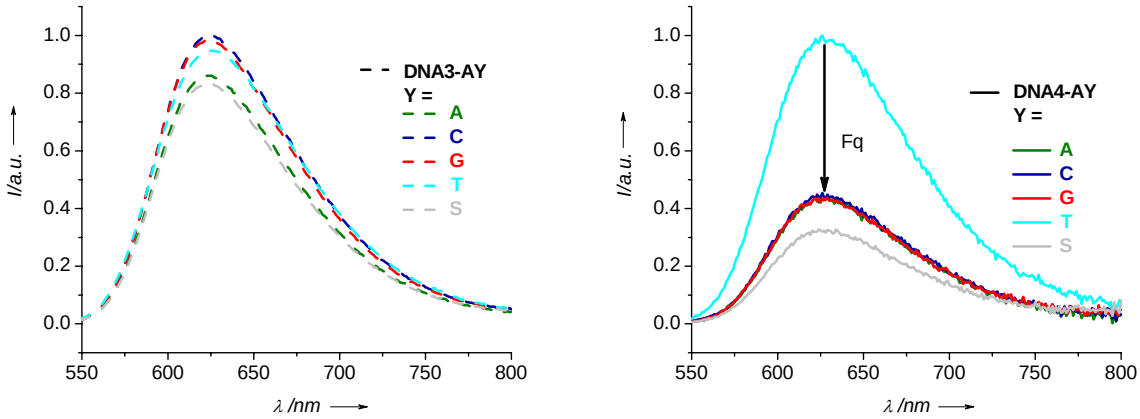

Figure S5. Fluorescence spectra of duplexes **DNA3-GY** and **DNA4-GY** (12.5 μM), Na-P_i-buffer (10 mM), pH=7, 25 °C, excitation at 530 nm.

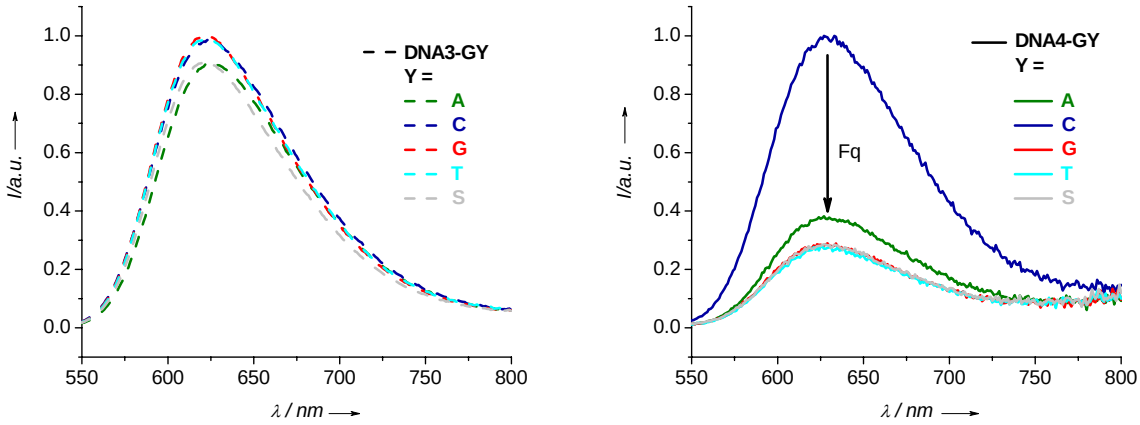

Figure S6. Fluorescence spectra of duplexes **DNA3-TY** and **DNA4-TY** (12.5 μM), Na-P_i-buffer (10 mM), pH=7, 25 °C, excitation at 530 nm.

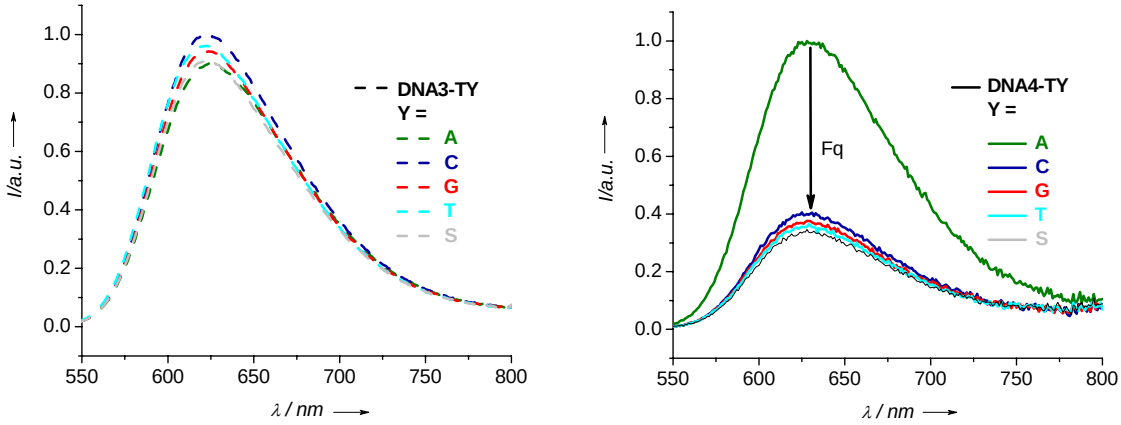

Figure S7. Fluorescence spectra of single-stranded oligonucleotides **ssDNA2-X** and **ssDNA4-X** together with the corresponding matched duplexes **DNA2-XY** and **DNA4-TY** (12.5 μM), Na-P_i-buffer (10 mM), pH=7, 25 °C, excitation at 530 nm.