Supplementary Material for PCCP

Photoinduced short-range electron transfer in DNA with fluorescent DNA bases: Lessons from ethidium as charge donor

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Supplementary Data

Content

Materials and Methods

^{1}H, ^{13}C, and the two dimensional NMR spectra were recorded at 300 K on a Bruker Avance 300 spectrometer in deuterated solvents. Chemical shifts are given in ppm relative to TMS. Mass spectra were measured in the central analytical faculty of the institute, ESI mass spectra on a ThermoQuest FinniganTSQ7000 in negative and positive ionization mode. Elemental analyses (C, H, N) were performed at in-house facilities. Analytical chromatography was performed on Merck silica gel 60 F_{254} coated aluminium foil. Flash chromatography was carried out on Merck silica gel 60 (40-63 μm). RP-C18 and RP-C5 (Discovery® Bio Wide Pore) analytical and semipreparative HPLC columns (300 Å) were purchased from Supelco. Chemicals were purchased from Aldrich, Alfa Aesar, ABI, Glen Research and Merck and used without further purification. Unmodified oligonucleotides were purchased from Metabion. Desalting of the DNA strands was performed on NAP-5 columns from GE Healthcare. Solvents were dried according to standard procedures; purified water with resistivity ≥ 18 MΩ cm^{-1} was used for preparation of buffer solvents. The fluorescence quantum yields were determined by the standard method with fluorescein in 1 N NaOH as reference (Φ_{Fl}= 0.98).[1]

Spectroscopic Measurements

All spectrometric measurements were performed at 20 °C in quartz glass cuvettes (1 cm) and using sodium phosphate buffer (10 mM). The absorption spectra and melting temperatures (2,5 μM DNA, 250 mM NaCl, 260 nm, 10 – 90 °C, scan speed 0.7 °C/ min, step width 0.5 °C) were recorded on a Varian Cary 100 spectrometer equipped with a 6x6 cell changer unit. Fluorescence was measured on a Jobin-Yvon Fluoromax 3 fluorimeter with a step width of 0.5 nm and an integration time of 0.2 s. Emission spectra of ethidium-modified DNA strands were recorded with a band-pass of 3 nm for both excitation and emission, whereas a band-pass of 2 nm was used for oligonucleotides containing a thiazole orange modification. All

spectra are corrected for Raman emission from the buffer solution. The fluorescence quantum yields (Φ_F) were determined by the standard method,[1] taking into account the refractive indices (n) of the solvents (eq S1):

$$\Phi_S = \Phi_R \cdot \frac{A_R \cdot n_S^2 \cdot F_S}{A_S \cdot n_R^2 \cdot F_R} \quad \text{(S1)}$$

The subscripts "*S*" and "*R*" refer to the sample and the reference dye, respectively. *A* is the extinction of the sample solution at the excitation wavelength; *F* is the emission integral over the area of interest. For thiazole orange-modified DNA strands quantum yields were measured using fluorescein in 1 N NaOH as reference (Φ_{Fl}= 0.98).[1]

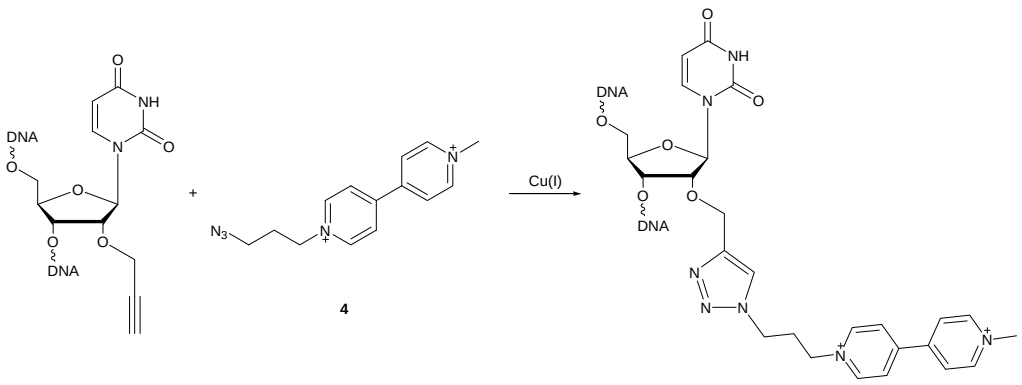
Spectrophotometric titrations. To avoid dilution of the analyte solutions, the titrant solutions contained DNA at a concentration of 408 μM DNA-bp as well as ethidium in the same concentration as in the titrated solution (30 μM) and sodium phosphate buffer (10 mM, pH7). Aliquots (500 μL) of the ethidium solution in sodium phosphate buffer (10 mM, pH7) were prepared in quartz glass cuvettes and titrated with the titrant solutions in 20 μL steps, and UV/Vis spectra were recorded (220-620 nm range). The titrations were finished when no further changes were observed in the absorption spectra. All spectrophotometric titrations were performed at least three times to ensure the reproducibility.

Spectrofluorimetric titrations. To avoid dilution of the analyte solutions, the titrant solutions contained DNA at a concentration of 51 μM DNA-bp as well as ethidium in the same concentration as in the titrated solution (3 μM) and sodium phosphate buffer (10 mM, pH7). Aliquots (500 μL) of the ethidium solution in sodium phosphate buffer (10 mM) were prepared in quartz glass cuvettes and titrated with the titrant solution in 20 μL steps. The isosbestic point (λ= 510 nm) as determined by the spectrophotometric titrations was chosen as excitation wavelength. Emission spectra of the titrations were recorded with a band-pass of 3

nm for both excitation and emission. The titrations were finished when no further changes were observed in fluorescence spectra. All spectrofluorimetric titrations were performed at least three times to ensure the reproducibility.

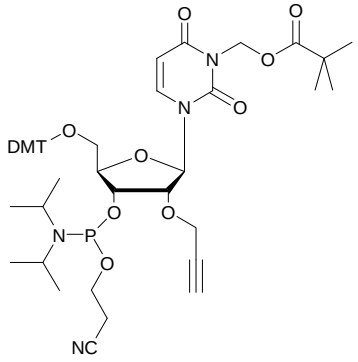
Strand displacement experiments. To avoid dilution of the analyte solutions, the titrant solution contained DNA at a concentration of 20 μM ssDNA as well as the semicomplementary ethidium and indole-modified duplex in the same concentration as in the titrated solution (2.5 μM) and sodium phosphate buffer (10 mM, pH7). Aliquots (500 μL) of the semicomplementary ethidium and indole-modified duplex solutions in sodium phosphate buffer (10 mM) were prepared in quartz glass cuvettes and titrated with the titrant solution in 10 μL steps. UV/Vis spectra were recorded in the range between 200 nm and 600 nm, for fluorescence spectra an excitation wavelength of 530 nm was chosen with a band pass of 3 nm for both excitation and emission. The titrations were finished when no further changes were observed in absorbance and fluorescence spectra.

Synthesis of methyl viologen-modified DNA. Methyl viologen-modified oligonucleotides were prepared using postsynthetic copper(I)-catalysed "click reaction" (Scheme S1).



Scheme S1: Postsynthetic DNA modification with Mv.

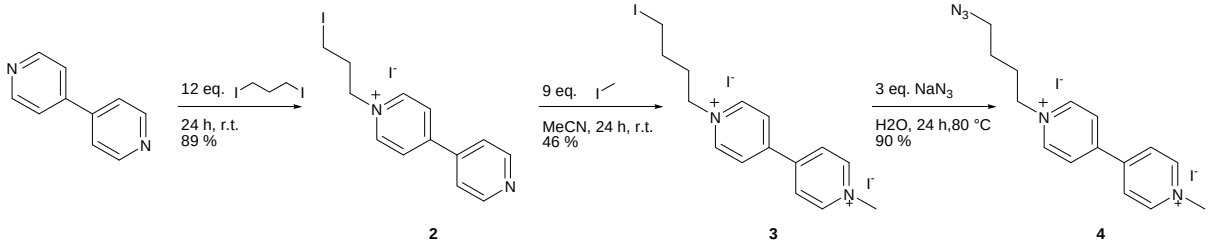
Synthesis of acetylene-modified uridine building block 1. The acetylene-modified uridine building block **1** (Figure S2) was synthesised according to published procedures.[2]



1

Fig. S1: Structure of DNA building block **1**.

Synthesis of the methyl viologen azide (4).



2 **3** **4**

Scheme S2: Synthesis of the methyl viologen azide (**4**).

Synthesis of *N*-(3-Iodopropyl)-4,4'-bipyridinium Iodide (2). 4,4'-bipyridine (1.18 g, 7.5 mmol) was suspended in diiodopropane (8 mL, 69.6 mmol) under a N_2 atmosphere. The suspension was sonicated for 1 h, and stirred at r.t. over night. Another aliquot of diiodopropane (3 mL, 26.1 mmol) was added and the reaction mixture was again stirred at r.t. over night. Et_2O (100 mL) was added and the resulting yellow participate was isolated, washed with Et_2O and dried under reduced pressure. For further purification, the product was recrystallized from a MeCN-Et_2O-mixture, washed with Et_2O and dried under reduced pressure to yield 3.02 g (89 %) of **2** as orange crystals. **^{1}H NMR** (DMSO-d_6, 300 MHz, 294.7 K): δ (ppm) 2.54 (m, 2 H, CH_2-2), 3.28 (t, 2H, CH_2-3, ^3J= 7 Hz), 4.70 (t, 2H, CH_2-1, ^3J= 7

Hz), 8.05 (dd, 2 H, 2 H3', ^3J= 5 Hz, ^4J= 2 Hz), 8.66 (d, 2 H, 2 H3, ^3J= 7 Hz), 8.87 (dd, 2 H, 2 H2', ^3J= 5 Hz, ^4J= 2 Hz), 9.24 (d, 2 H, 2 H2, ^3J= 7 Hz). **^{13}C NMR** (DMSO-d_6, 150 MHz, 294.7 K): δ (ppm) 0.0 (CH_2-CH_2-*C*H_2-I) 32.6 (CH_2-*C*H_2-CH_2-I), 59.5 (*C*H_2-CH_2-CH_2-I), 120.5 (2 C3'), 124.0 (2 C3), 139.4 (C4'), 144.1 (2 C2'), 149.5 (2 C2), 150.9 (C4). **ESI-MS** m/z (%): 324.9 (100) [$M^{\cdot+}$].

Synthesis of *N*-(3-Iodopropyl)-*N*'-methyl-4,4'-bipyridinium Iodide (3). Under a N_2 atmosphere, iodomethane (3 mL, 48.2 mmol) was added to a suspension of compound **2** (2.54 g, 5.62 mmol) in dry MeCN (70 mL). The reaction mixture was stirred at r.t. over night and subsequently concentrated to dryness. The residue was extensively washed with hot EtOH and dried under reduced pressure to yield 1.52 g (46 %) of a red solid. **^{1}H NMR** (DMSO-d_6, 300 MHz, 294.7 K): δ (ppm) 2.56 (m, 2 H, C*H*$_2$-2), 3.28 (t, 2H, C*H*$_2$-3, ^3J= 7 Hz), 4.44 (s, 3H, C*H*$_3$), 4.74 (t, 2H, C*H*$_2$-1, ^3J= 7 Hz), 8.78 (d, 2 H, 2 H3', ^3J= 7 Hz), 8.82 (d, 2 H, 2 H3, ^3J= 7 Hz), 9.30 (d, 2 H, 2 H2', ^3J= 7 Hz), 9.38 (d, 2 H, 2 H2, ^3J= 7 Hz). **^{13}C NMR** (DMSO-d_6, 150 MHz, 294.7 K): δ (ppm) 0.0 (CH_2-CH_2-*C*H_2-I) 32.7 (CH_2-*C*H_2-CH_2-I), 46.8 (*C*H_3), 60.1 (*C*H_2-CH_2-CH_2-I), 124.8 (2 C3), 125.3 (2 C3'), 144.7 (2 C2), 145.4 (2 C2'), 146.7 (C4), 147.3 (C4'). **ESI-MS** m/z (%): 171.0 (18) [M-C_3H_6I]$^+$, 338.9 (48) [M-H$^+$]$^+$, 339.9 (100) [$M^{\cdot+}$]. **EA** calculated for $C_{14}H_{17}I_3N_2$ (349.21): C 28.31; H 2.88; N 4.72. found: C 28.31; H 2.94; N 4.64.

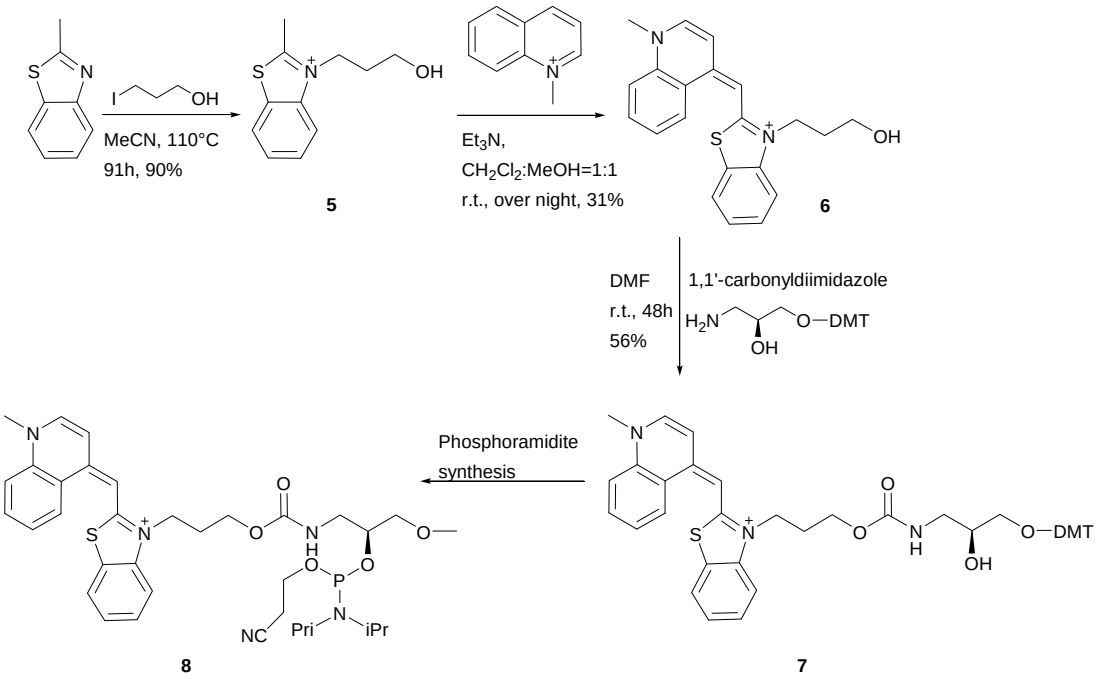
Synthesis of *N*-(3-Azidopropyl)-*N*'-methyl-4,4'-bipyridinium Iodide (4). Compound **3** (0.30 g, 0,5 mmol) and sodium azide (0.13 g, 1.5 mmol) were dissolved in water (0.5 mL). The reaction mixture was stirred at 80 °C over night and subsequently concentrated to dryness. The residue was washed with MeOH (0.5 mL) and dried under reduced pressure to yield 0.23 g (90 %) of a brown solid. **ESI-MS** m/z (%): 255.0 (60) [$M^{\cdot+}$], 300.0 (100) [M^{2+}+$HCOO^-$]$^+$, 368.0 (20) [M^{2+}+TFA^-]$^+$.

Preparation of uridine-modified DNA. Oligonucleotides were prepared on an Expedite 8909 Synthesizer from Applied Biosystems (ABI) using standard phosphoramidite chemistry. Reagents and controlled pore glass (CPG; 1 μmol) were purchased from ABI and Glen Research. Acetylene-modified uridine was introduced into DNA by using standard coupling conditions. The concentration of the building block was increased to 0.1 M. After preparation, the trityl-off oligonucleotide was cleaved from the resin and deprotected by treatment with conc. NH_4OH at r.t. for 24 hours.

Postsynthetic modification and DNA purification. The azide **4** (114 μL, 10 mM), Cu(I) (17 μL, 100 mM), TBTA (34 μL, 100 mM), each in DMSO/^{t}BuOH = 3:1, and sodium ascorbate (25 μL, 400 mM) in H_2O was added to the oligonucleotide (1 μmol). The reaction mixture was vortexed, shaken over night at r.t., and then evaporated to dryness using a speedvac. Sodium acetate (100 μL, 0.15 mmol) was added and the mixture stored for 1 h at r.t. EtOH (1 mL) was added and the mixture vortexed and stored in the freezer (-20 °C) overnight. The suspension was centrifugated (13 000 rpm, 15 min) and the supernatant removed. The pellet was washed twice with EtOH (500 μL), was then dissolved in H_2O (500 μL) and desalted using a NAP-5 column. The oligonucleotide was dried and purified by HPLC on a semipreparative RP-C5 column (300 Å, Supelco) using the following conditions: A = NH_4OAc buffer (50 mM), pH = 6.5; B = MeCN, gradient 3- 12 % B over 50 min., flow rate 2.5 mL/min, UV/Vis detection at 260 and 310 nm. The purified oligonucleotide was identified by **ESI-MS** m/z: **DNA2a** calcd: 5444, found: 1360 $[M^{2+}\text{-}6H^{+}]^{4-}$,1814 $[M^{2+}\text{-}5H^{+}]^{3-}$. The oligonucleotide was quantified by its absorbance at 260 nm using ε_{260nm}= 34100 M^{-1} cm^{-1} for methyl viologen dU.

Synthesis of thiazole-orange modified DNA. Thiazole orange-modified DNA strands were prepared using standard phosphoramidite chemistry.

Synthesis of DNA building block 8.



Scheme S3: Synthesis of the thiazole orange DNA building block **8**.

Synthesis of 2-methyl-3-propanoyl-benzothiazolium iodide (5). In a nitrogen atmosphere, 2-methyl-benzothiazole (13.7 mL, 107.5 mmol) and 3-iodo-1-propanole (10.0 g, 53.8 mmol) were dissolved in MeCN (30 mL) and the solution was refluxed for 91 h. The reaction mixture was allowed to cool to r.t. and kept in the fridge over night. The precipitate was collected, washed three times with cold Et_2O (15 mL), and dried under reduced pressure to yield 30.8 g (90.0 %) of a white solid. **^{1}H NMR** (DMSO-d_6, 300 MHz, 294.7 K): δ (ppm) 2.06 (m, 2H, -CH_2-), 3.22 (s, 3H, Me), 3.53 (m, 2H, -CH_2-O), 4.78 (t, 2H, ^3J= 7 Hz, NCH_2-), 4.83 (m, 1H, O*H*), 7.93 – 7.78 (m, 2H, arom.), 8.46 – 8.29 (m, 2H, arom.). **^{13}C NMR** (DMSO-d_6, 150 MHz, 294.7 K): δ (ppm) 16.7 (2-Me), 30.4 (propyl), 45.8, 57.3, 116.7 (benzothiazole arom.), 124.6, 128.0, 129.0, 129.3, 140.8, 177.2. **ESI-MS** m/z (%): 208.1 (100) $[M]^+$.

Synthesis of 2-[(1,4-dihydro-1-methyl-chinolin-4-methylyliden)-]-3-propanoyl benzothiazolium iodide (6). Compound **5** (1.15 g, 3.5 mmol) and *N*-methylchinoline (0.93 g, 3.5 mmol) were dissolved in CH_2Cl_2/MeOH (1:1 v:v; 10 mL) in a nitrogen atmosphere. Dry Et_3N (1.2 mL, 8.58 mmol) was added resulting in an immediate colour change to deep red. The reaction mixture was refluxed over night and subsequently evaporated to dryness. The crude product was purified by flash column (SiO_2, 1-5 % MeOH in CH_2Cl_2) yielding 0.82 g (71 %) of a dark red solid. **TLC** (CH_2Cl_2/MeOH 9:1) R_f = 0.27. **^{1}H NMR** (DMSO-d_6, 300 MHz, 294.7 K): δ (ppm) 2.00 (m, 2H, -CH_2-), 3.58 (m, 2H, -CH_2-O), 4.18 (s, 3H, N-Me), 4.63 (m, 2H, N-CH_2-), 5.05 (t, 1H, ^3J= 5 Hz, OH), 7.05 (s, 1H, Olefin-H), 7.44 – 7.37 (m, 2H, arom.), 7.64 – 7.58 (m, 1H, arom.), 7.79 – 7.74 (m, 2H, arom.), 8.09 – 7.99 (m, 3H, arom.), 8.43 (m, 1H, arom.), 8.75 (m, 1H, arom.). **^{13}C NMR** (DMSO-d_6, 150 MHz, 294.7 K): δ (ppm) 30.1 (propyl), 42.3, 45.6 (N-Me), 57.3 (propyl), 87.4 (thiazole orange), 107.8, 112.7, 118.2, 122.9, 123.8, 124.0, 124.4, 125.2, 126.9, 128.1, 133.1, 138.0, 139.9, 145.0, 148.6, 159.2. **ESI-MS** m/z (%): 349.0 (100) $[M]^+$. **FAB-HR-MS** m/z (%) calculated for $C_{11}H_{14}NOS^+$: 349.1375; found: 349.1380.

Synthesis of (3-(bis-(4-methoxy-phenyl)-phenyl-methoxy)-2-hydroxy-propylcarbamic acid)-[4-[(1,4-dihydro-1-methylquinoline-4-ylidene)-methyl]-benzothiazolium iodide]-3-propyl ester (7). Compound **6** (434 mg, 0.91 mmol) was dissolved in DMF (5 mL) and 1,1'-carbonyldiimidazole (195 mg, 1.0 mmol) was added. The solution was stirred at r.t. for 3 h. 3-((4,4'-dimethoxytrityl)-2-hydroxy)-propylamine (395 mg, 1.0 mmol) was added and the solution was stirred for 48h at r.t. and subsequently evaporated to dryness. The crude product was purified by flash column (SiO_2, 0 – 5 % MeOH in CH_2Cl_2) yielding 456 mg (56 %) of a dark red solid. **TLC** (CH_2Cl_2/MeOH 9:1) R_f = 0.40. **^{1}H NMR** (DMSO-d_6, 300 MHz, 294.7 K): δ (ppm) 2.09 (m, 2H, -CH_2-), 2.86 (m, 1H, -CH_2-ODMT), 2.96 (m, 1H, -CH_2-ODMT), 3.16 (m, 1H, NH-CH_2-), 3.30 (m, 1H, NH-CH_2-), 3.33 (s, 1H,

N*H*), 3.70 (s, 6H, 2 OMe), 3.73 (s, 1H, -C*H*-OH), 4.10 (m, 2H, -CH_2-O), 4.19 (s, 3H, N-Me), 4.64 (m, 2H, N-CH_2-), 4.98 (d, 1H, ^3J= 5 Hz, O*H*), 6.92 – 6.84 (m, 4H, arom. DMT), 7.23 (m, 7H, arom. DMT), 7.34 (m, 2H, arom. DMT), 7.37 (m, 2H, arom. TO), 7.59 (m, 1H, arom. TO), 7.80 – 7.68 (m, 2H, arom. TO), 8.09 – 7.96 (m, 3H, arom. TO), 8.64 (m, 1H, arom. TO), 8.75 (m, 1H, arom. TO). **^{13}C NMR** (DMSO-d_6, 150 MHz, 294.7 K): δ (ppm) 28.8 (propyl), 42.4 (propyl), 54.9 (OMe), 65.6 (*C*H_2O), 68.6, 85.1 (O-*C*-(PhOMe)$_2$Ph), 112.9, 123.8, 126.4, 126.9, 127.6, 128.3, 128.6, 129.6, 130.2, 131.5, 133.0, 135.6, 137.9, 139.8, 145.0, 148.8, 157.9, 166.8. **ESI-MS** m/z (%): 768.3 (100) $[M]^+$. **FAB-HR-MS** m/z (%) calculated for $C_{46}H_{46}N_3O_6S^+$: 768.3107; found: 768.3096.

Synthesis of DNA building block 8. Compound **7** (60 mg, 0.067 mmol) was dissolved in dry CH_2Cl_2 (4 mL) in a nitrogen atmosphere. Dry di*iso*propylamine (35 μL, 0.2 mmol) and 2-cyanoethyl-*N*,*N*-di*iso*propylchlorophosphoramidite (21 μL, 0.094 mmol) were added. TLC showed complete conversion after 60 min. stirring at r.t. The reaction mixture was washed with freshly prepared sat. aq. $NaHCO_3$, dried over Na_2SO_4, and evaporated to dryness yielding 65 mg (90 %) of a dark red solid. The product was dissolved in dry MeCN (0.6 mL) and applied directly for automated DNA synthesis. **TLC** (CH_2Cl_2:MeOH = 9:1) R_f = 0.50. **^{31}P-NMR** (DMSO-d_6, 121 MHz, 294.7 K): δ (ppm) 149.06, 149.59.

Preparation and purification of thiazole-orange modified DNA. Oligonucleotides were prepared on an Expedite 8909 Synthesizer from Applied Biosystems (ABI) using standard phosphoramidite chemistry. Reagents and controlled pore glass (CPG; 1 μmol) were purchased from ABI and Glen Research. The synthesis of thiazole orange-modified DNA oligonucleotides was performed using a modified protocol. Activator solution (0.45 M tetrazole in MeCN) was pumped simultaneously with the building block (0.1 M in MeCN). The coupling time was extended to 61 minutes with an intervening step after 30.8 min for

washing and refreshing the activator/phosphoramidite solution in the CPG vial. The CPG vial was flushed with dry MeCN after the coupling. After preparation, the trityl-off oligonucleotide was cleaved from the resin and deprotected by treatment with conc. NH_4OH at room temperature for 36 hours. The modified oligonucleotides were purified by HPLC on a semipreparative RP-C18 column (300Å, Supelco) using the following conditions: A= NH_4OAc buffer (50 mM), pH = 6.5; B= MeCN; gradient 0-20% B over 50 min, flow rate 2.5 mL/min, UV/Vis detection at 260 and 512 nm. The purified oligonucleotides were identified by **ESI-MS** m/z: **DNA3a** calcd: 5406, found: 1351 $[M-5H^+]^{4-}$,1802 $[M-4H^+]^{3-}$; **DNA3b** calcd: 5407, found: 1351 $[M-5H^+]^{4-}$,1802 $[M-4H^+]^{3-}$; **DNA4a** calcd: 5406, found: 1351 $[M-5H^+]^{4-}$,1802 $[M-4H^+]^{3-}$; **DNA4b** calcd: 5407, found: 1351 $[M-5H^+]^{4-}$,1802 $[M-4H^+]^{3-}$. The oligonucleotides were quantified by their absorbance at 260 nm on a Varian Cary 100 spectrometer (TO does not absorb light at 260 nm).

DNA-Titration Experiments

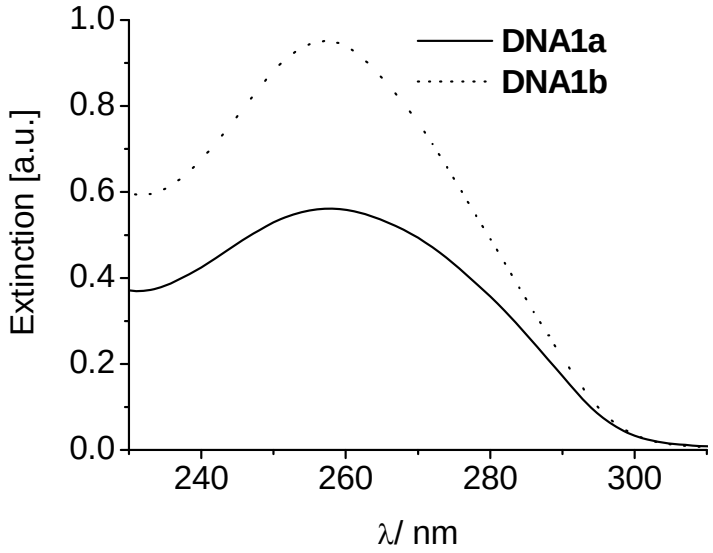


Fig. S2: Absorbance spectra of indole-modified **DNA1a** (2.5 µM) and unmodified **DNA1b** (2.5 µM) in sodium phosphate buffer (10 mM, pH 7, 20°C).

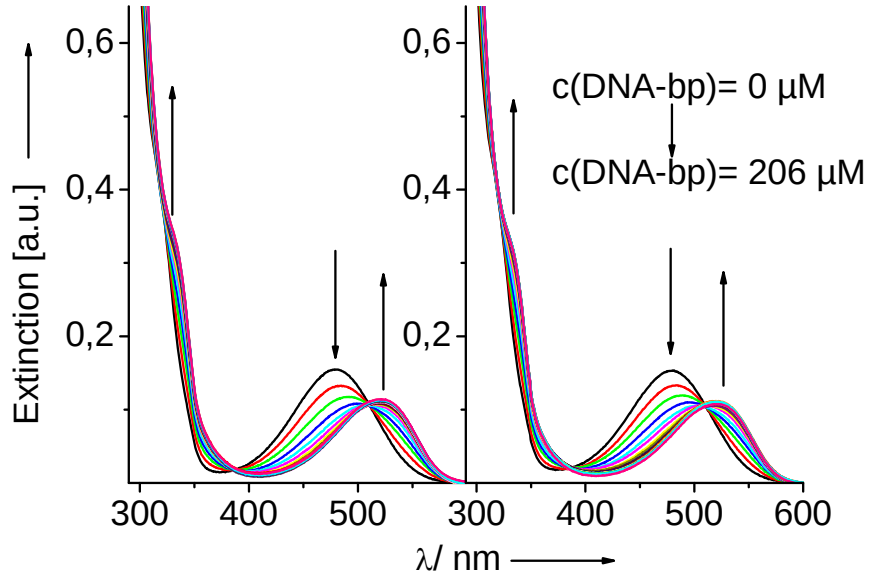


Fig. S3: Spectrophotometric titration of **Et** (c= 30 µM) with **DNA1a** (left) or **DNA1b** (right) in sodium phosphate buffer (10 mM, pH 7, 20°C). Arrows indicate changes in the intensity of the bands upon increasing DNA concentrations.

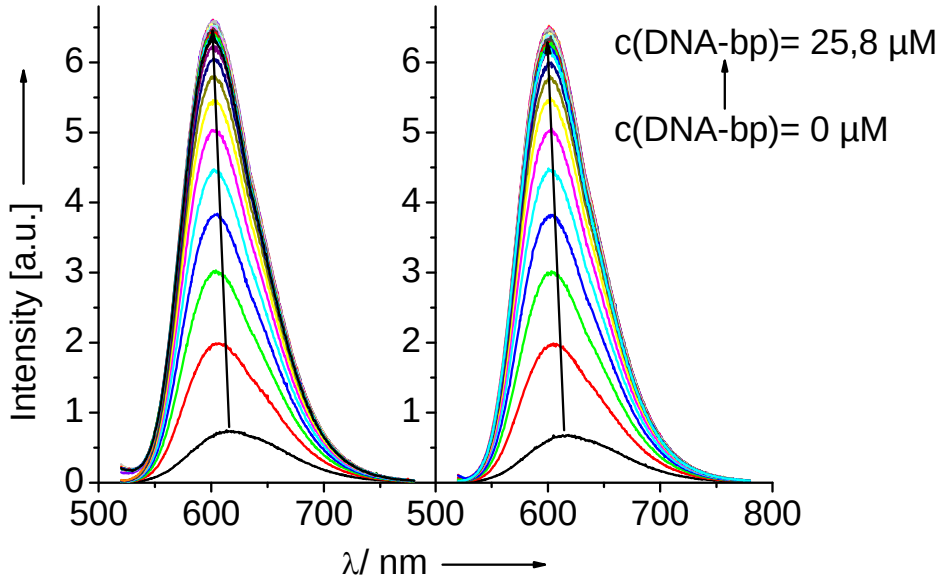


Fig. S4: Spectrofluorimetric titration of **Et** (c= 3 µM) with **DNA1a** (left) or **DNA1b** (right) in sodium phosphate buffer (10 mM, pH 7, 20°C, λ_{exc}= 510 nm). Arrows indicate changes in the intensity of the bands upon increasing DNA concentrations.

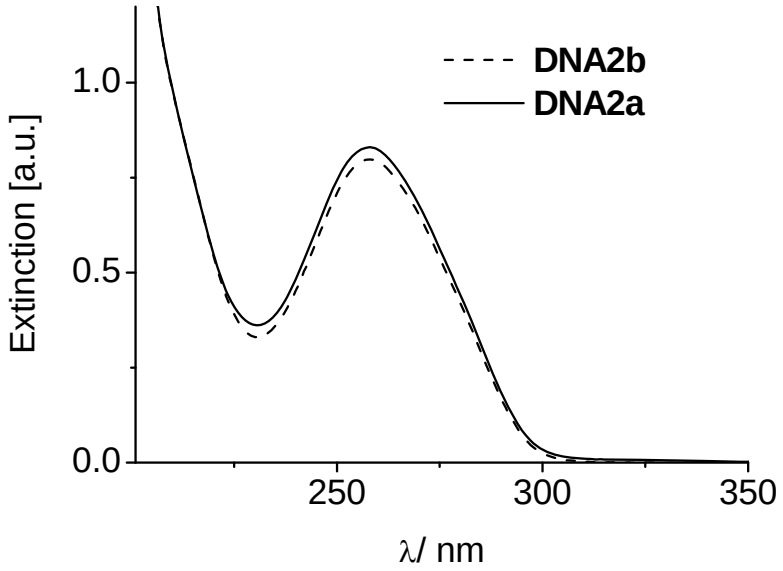


Fig. S5: Absorbance spectra of methyl viologen-modified **DNA2a** and unmodified **DNA2b**; 2.5 µM in sodium phosphate buffer (10 mM, pH 7, 20°C).

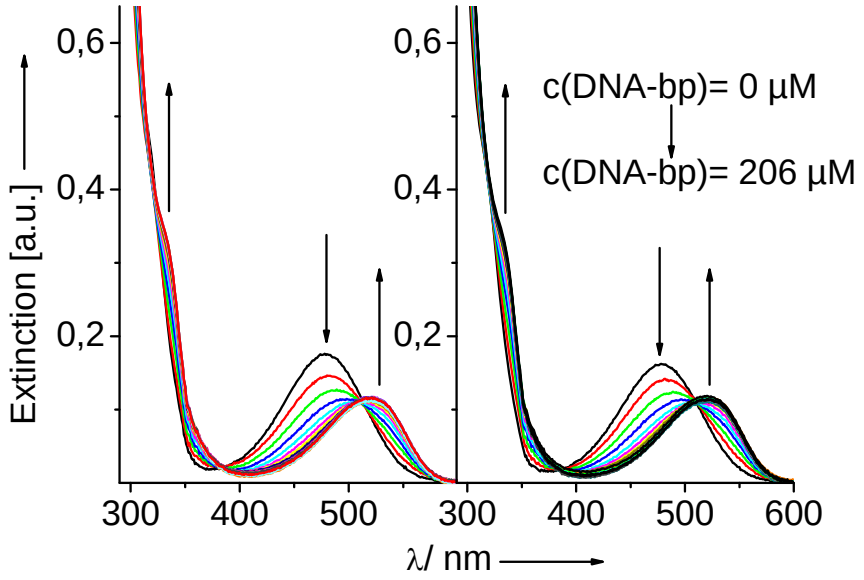


Fig. S6: Spectrophotometric titration of **Et** (c= 30 µM) with **DNA2a** (left) or **DNA2b** (right) in sodium phosphate buffer (10 mM, pH 7, 20°C). Arrows indicate changes in the intensity of the bands upon increasing DNA concentrations.

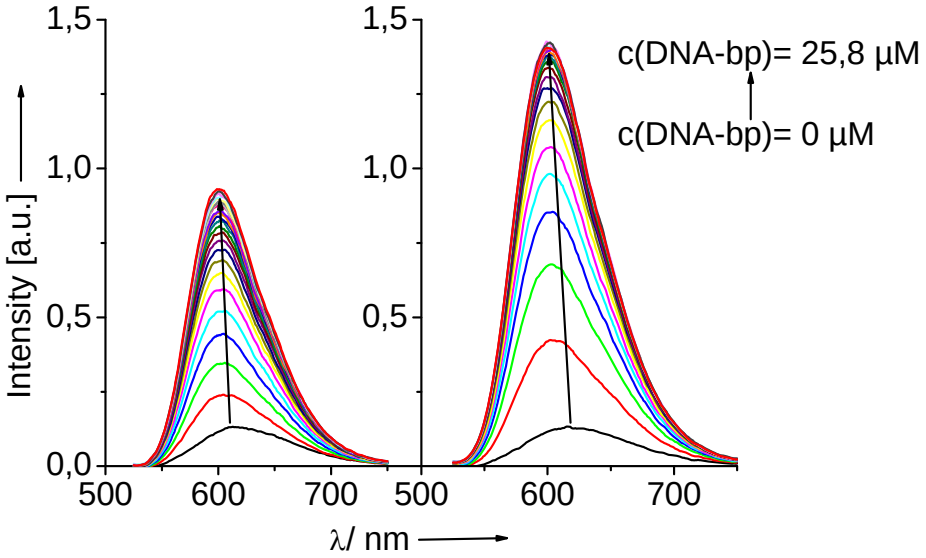


Fig. S7: Spectrofluorimetric titration of **Et** (c= 3 µM) with **DNA2a** (left) or **DNA2b** (right) in sodium phosphate buffer (10 mM, pH 7, 20°C, λ_{exc}= 510 nm). Arrows indicate changes in the intensity of the bands upon increasing DNA concentrations.

DNA-Duplex	Modification	T_m [°C]	ΔT_m [°C]
DNA1a	In	53.4	-18.3
DNA1b	unmodified	71.7	-
DNA2a	Mv	61.3	-1.2
DNA2b	unmodified	62.5	-

Table S1: Melting temperatures of duplexes **DNA1a - DNA2b** (2.5 µM in 10 mM sodium phosphate buffer, pH 7, 250 mM NaCl).

Thiazole orange-modified DNA

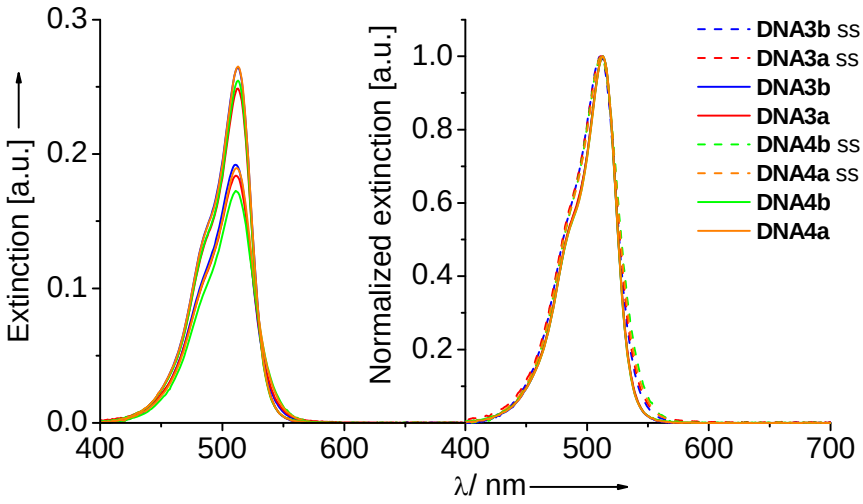


Fig. S8: Absorbance spectra of **DNA3a** – **DNA4b**. Left: 2.5 µM in sodium phosphate buffer (10 mM, pH 7, 20°C); right: normalized absorbance spectra (ss: single stranded; ds: double stranded).

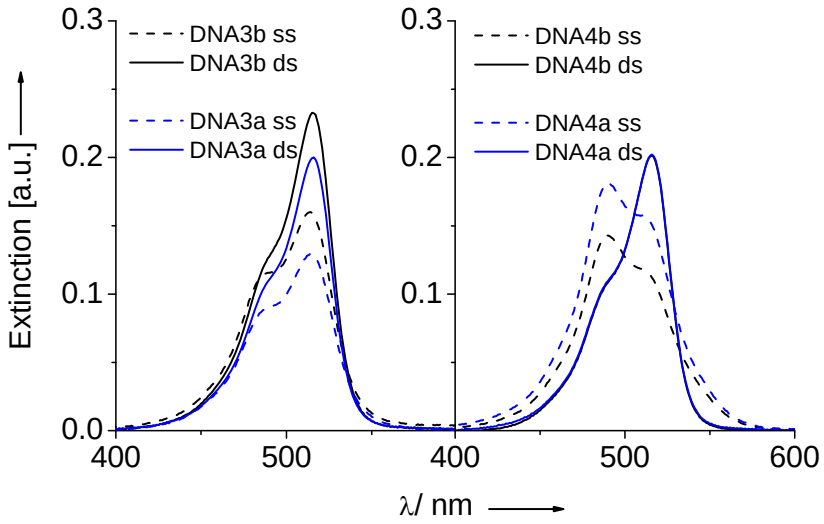


Fig. S9: Absorbance spectra of **DNA3a** – **DNA4b** (2.5 µM) in sodium phosphate buffer (10 mM, pH 7, 250 mM NaCl, 20°C). The absorption changes that are observed in case of **DNA4b** (ss) and **DNA4a** (ss) can be attributed to an excitonic interactions between two **To** chromophores due to partial self-complementarity of the single strands., as published recently by our group.[3]

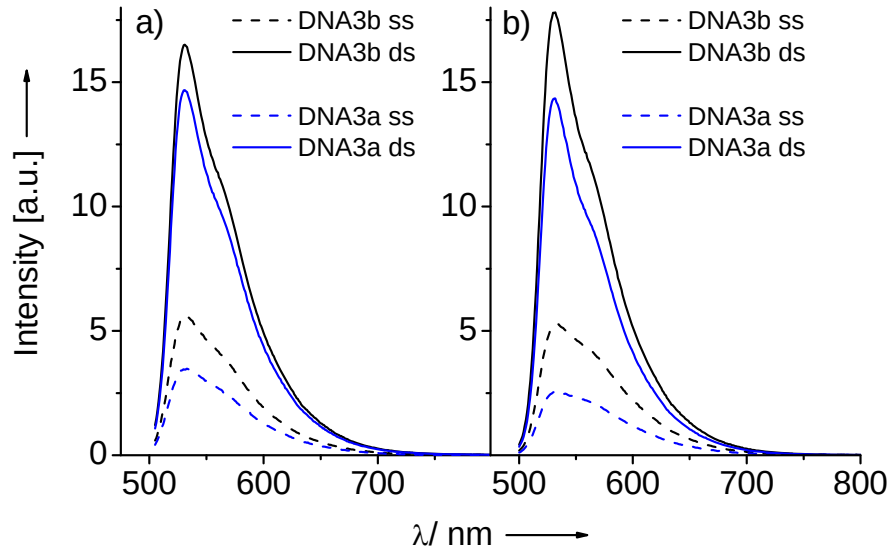


Fig. S10: Fluorescence spectra (λ_{exc}= 490 nm) of the **DNA3a** and **DNA3b**: a) 2.5 µM in sodium phosphate buffer (10 mM, pH 7, 20°); b) additional 250 mM NaCl.

DNA-Duplex	Modification	Quantum yield Φ	T_m [°C]
DNA3b	To*	0.22	67.5
DNA4b	To*	0.24	65.5
DNA3c	To'$^{\pm}$	0.10	66.2
DNA4c	To'$^{\pm}$	0.10	65.3

* incorporated into DNA via its benzothiazole nitrogen, without acceptor
$^{\pm}$ incorporated into DNA via its quinoline nitrogen, without acceptor

Table S2: Quantum yields (20 °C) and melting temperatures of thiazole orange-modified duplexes **DNA3b** – **DNA4c** (2.5 μM in 10 mM sodium phosphate buffer, pH 7, 250 mM NaCl).

Ethidium and nitroindole-modified DNA

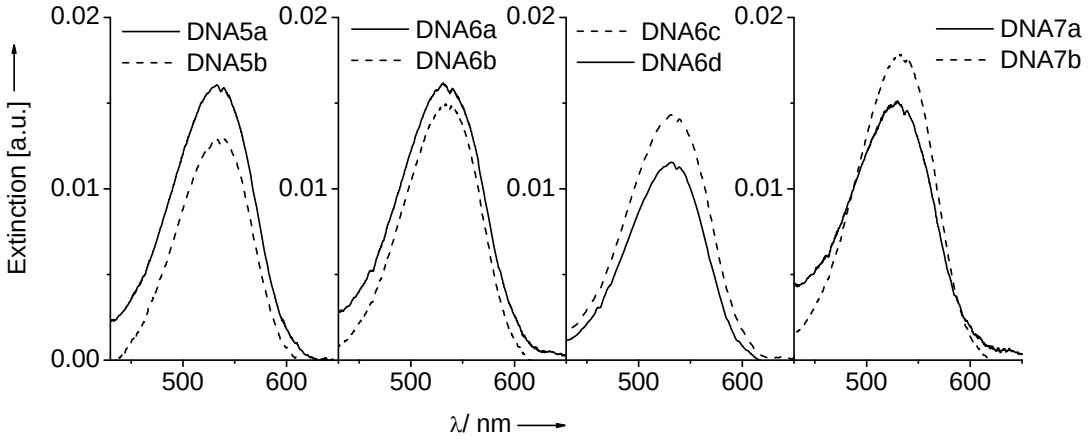


Fig. S11: Absorbance spectra of the **DNA5a** – **DNA7b** (2.5 μM) in sodium phosphate buffer (10 mM, pH 7, 20°).

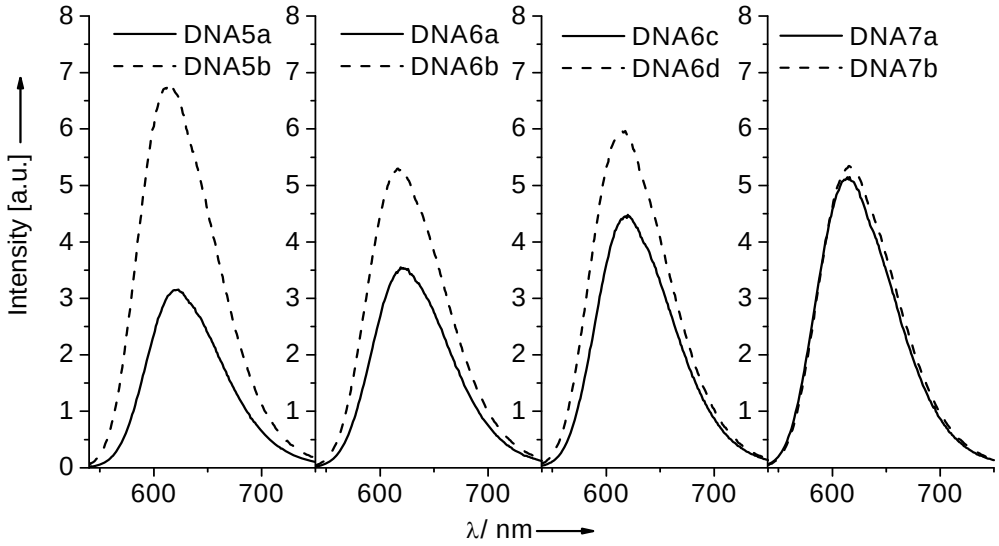


Fig. S12: Fluorescence spectra (λ_{exc}= 530 nm) of the **DNA5a** – **DNA7b** (2.5 μM) in sodium phosphate buffer (10 mM, pH 7, 20°). Emission spectra were corrected against optical density.

DNA-Duplex	τ_1 [ns]	A_1 [%]	τ_2 [ns]	A_2 [%]	τ [ns]	τ [ns]*
DNA5a	0.818 ± 0.01	0.226	12.4 ± 0.01	0.774	9.81	9.45
DNA5b	2.27 ± 0.05	0.184	20.2 ± 0.05	0.816	16.9	16.6
DNA6a	1.21 ± 0.02	0.204	13.6 ± 0.02	0.796	11.1	12.8
DNA6b	0.707 ± 0.02	0.250	17.4 ± 0.05	0.750	13.3	15.5
DNA7a	1.07 ± 0.02	0.148	18.2 ± 0.03	0.852	15.7	15.7
DNA7b	0.790 ± 0.02	0.131	18.9 ± 0.05	0.869	16.5	16.7

* Fluorescence lifetimes from Ref.[4] An abasic site was used opposite to ethidium in the counterstrand.

Table S3: Fluorescence lifetimes of duplexes **DNA5a** – **DNA7a** and **DNA5b** – **DNA7b** (2.5 μM in 10 mM sodium phosphate buffer, pH 7, 250 mM NaCl, 20 °C). C was used as counterbase to ethidium.

Ethidium and indole-modified DNA

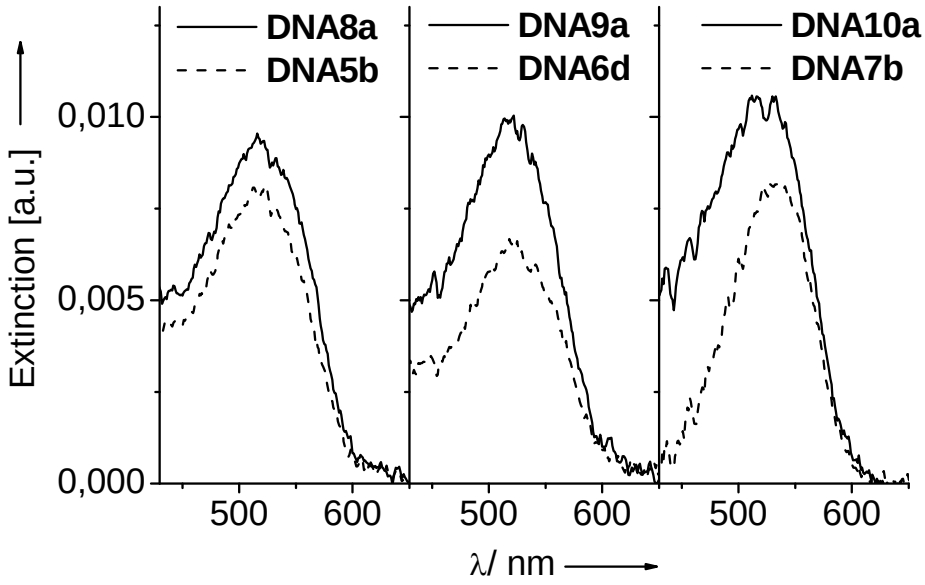


Fig. S13: Absorbance spectra of the **DNA8a** – **DNA10a** and **DNA5b** – **DNA7b** (5 μM) in sodium phosphate buffer (10 mM, pH 7, 250 mM NaCl, 20°).

DNA-Duplex	Modification	T_m [°C]	ΔT_m [°C]
DNA8a	Et/In'	53.5	-13.5
DNA5b	Et	67.0	-
DNA9a	Et/In'	53.2	-16.0
DNA6d	Et	69.2	-
DNA10a	Et/In'	47.1	-15.3
DNA7b	Et	62.4	-

Table S4: Melting temperatures of duplexes **DNA8a** – **DNA10a** and **DNA5b** – **DNA7d** (2.5 μM in 10 mM sodium phosphate buffer, pH 7, 250 mM NaCl).

Strand displacement experiments

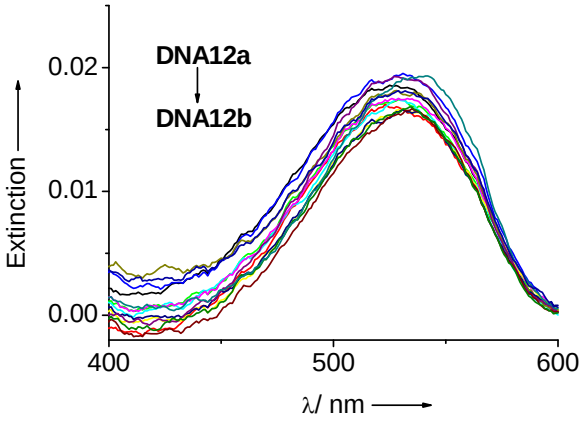


Fig. S14 Absorbance spectra for the strand displacement experiments. The semicomplementary duplex **DNA12a** (2.5 µM dsDNA in 10 mM Na-P_i buffer, pH7, 20 °C) is titrated with ssDNA (20 µM ssDNA and 2.5 µM dsDNA in 10 mM Na-P_i buffer, pH7, 20 °C) that is perfectly complementary to the ethidium-modified strand of **DNA12a** (up to 2 equivalents ssDNA; ss = single stranded, ds = double stranded).

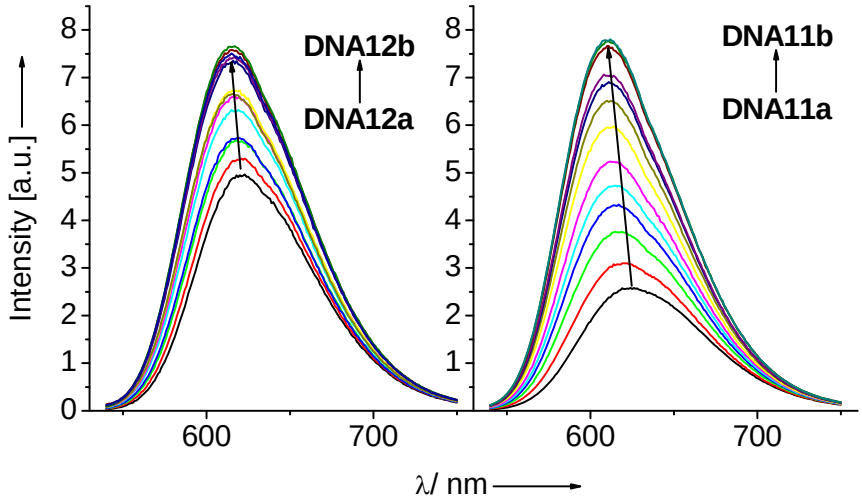


Fig. S15 Fluorescence titration curves for the strand displacement experiments (λ_{exc}= 530 nm). The semicomplementary duplexes **DNA11a** and **DNA12a** respectively (2.5 µM dsDNA in 10 mM Na-P_i buffer, pH7, 20 °C) are titrated with ssDNA (20 µM ssDNA and 2.5 µM dsDNA in 10 mM Na-P_i buffer, pH7, 20 °C) that is perfectly complementary to the ethidium-modified strand of **DNA11a** or **DNA12a** (ss = single stranded, ds = double stranded). Arrows indicate changes in the intensity of the bands upon increasing ssDNA concentrations (up to 2 equivalents ssDNA).

References

1. G. A. Crosby and J. N. Demas, *J. Phys. Chem.* , 1971, **75**, 991-1024.
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3. S. Berndl and H.-A. Wagenknecht, *Angew. Chem. Int. Ed.*, 2009, **48**, 2418-2421.
4. L. Valis, Q. Wang, M. Raytchev, I. Buchvarov, H.-A. Wagenknecht and T. Fiebig, *Proc. Natl. Acad. Sci. USA*, 2006, **103**, 10192-10195.