Supplementary Material (ESI) for Chemical Communications

Electron transfer through a stable phenanthrenyl pair in DNA

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Electronic supporting information

Experimental section

Reagents. Phenanthrenyl [1] and Py-dU[2] phosphoramidites were synthesized as described. The phosphoramidites of the natural nucleosides, Br-dU and fluorescein were from *Glen Research*. All other reagents were purchased from *Sigma-Aldrich* and were of highest purity available. Piperidine was distilled before use and stored at -20°C.

DNA synthesis and purification. All oligonucleotides were synthesized on a 1 μmol scale using a *Polygen* DNA synthesizer (Vers. 03) in the trityl-off mode. Except for 5-(Ethylthio)-1*H*-tetrazole (0.25 M in CH_3CN) as activator, standard reagents and solutions were used. For the dPhen phosphoramidite the coupling time was increased to 4 min. Coupling yields of >99% (trityl assay) were obtained. Oligonucleotides were cleaved from solid support and deprotected in 32% aqueous ammonia for 20 h at RT in the dark. Fluorescein-labeled strands were purified by preparative gel-electrophoresis, desalted by drop dialysis for 2 h using Millipore membranes (V-series), lyophilized and re-dissolved in de-ionized water. All other

oligonucleotides were purified by RP-HPLC (Nucleosil 300-S C_{18}, *Macherey-Nagel*; A = 0.1 M triethylammonium acetate in H_2O, pH 7.0; B = 0.1 M triethylammonium acetate in CH_3CN/H_2O 4:1, pH 7.0; linear gradients of B in A; detection wavelength: 260 nm), lyophilized and re-dissolved in de-ionized water. Purified oligonucleotides were quantified by UV (260 nm) on a *NanoDrop* ND-1000 UV-Vis spectrophotometer using ε (260 nm)=38'100 M^{-1} cm^{-1} for dPhen,[1] ε (260 nm)=14'600 M^{-1} cm^{-1} for Py-dU,[2] ε (260 nm)=20'900 M^{-1} cm^{-1} for fluorescein[3] and ε (260 nm)=7'000 M^{-1} cm^{-1} for Br-dU[4]. The synthesis and analytical data are summarized in Table S1.

Strand cleavage experiments. Duplexes (4 μM duplex, 10 mM Na-P_i-buffer, 150 mM NaCl, pH 7.0) were prepared by heating equimolar solutions of the single strands to 70°C for 10 min in the dark and cooling to room temperature during 10 min. Samples were deoxygenated by bubbling Ar for 20 min through the solution and then irradiated in quartz glass cuvettes (1 cm). Cuvettes with freshly prepared duplexes were placed into a container made of window glass (wall thickness 3 mm), which served as a cut-off filter for wavelengths below 300 nm. The container was water cooled to maintain the temperature of the irradiated sample at +14-16°C. The samples were irradiated using a HPK 125W Hg, Ba/Sr lamp (125 W, *Heraeus*). The distance from the lamp to the front surface of cuvettes was kept constant throughout all experiments (13 cm). Aliquots (20 μL) of the sample solution (300 μL) were taken after 0, 5, 10, 15, 20, 30, 40, 60 and 90 min of irradiation, placed into sterile containers and stored protected from light at room temperature. After withdrawing the last aliquot, all samples were treated with piperidine (2.2 μL) and subsequently heated to 90°C for 30 min, cooled to RT and lyophilized. Loading buffer (5 μL, 900 μL of formamide, 22 μL of 0.5 M EDTA solution pH 8.0 and 78 μL of de-ionized water) was added and samples were treated with ultrasound for 2 min at RT. After sonication samples were heated to 60°C for 10 min, cooled to RT and loaded (5 μL) onto a gel (170 x 115 x 0.8 mm; 19:1 acrylamide – bis-acrylamide, 20% w/v, 7

M urea, 100 mM Tris-borate and 20 mM EDTA buffer, pH 8.3). Gels were run at 250 V for 2.5 h and analyzed using a *Fuji* FLA-3000 image plate reader. Band intensities were quantified with Advanced Image Data Analyzer software (*Raytest*). All experiments were performed in duplicate and the relative deviation of data points were determined to be less than 0.1%.

UV-melting curve analysis. Thermal denaturation experiments (1.2 μM duplex, 10 mM Na-Pi-buffer, 150 mM NaCl, pH 7.0) were carried out on a *Varian* Cary 100 UV-Vis spectrophotometer. Absorbances were monitored at 260 nm and the heating rate was set to 0.5°C/min. A cooling-heating-cooling cycle in the temperature range 10-60°C was applied. T_m values (λ = 260 nm) were defined as the maximum of the first derivative of the melting curve. All T_m values are the average of at least two independent experiments.

Table S1. Synthesis and mass-spectrometric data of Phen containing oligonucleotides.

Oligonucleotide sequence	Yield,[a] (O.D.^{260})	ESI$^-$-MS (found)	ESI$^-$-MS (calcd)
5'-Flu-d(CGCAT-^{Br}U-T-Py-ATCGC)	8.9	4690.0	4689.1
5'-Flu-d(CGCAT-^{Br}U-Phen-Py-ATCGC)	9.2	4741.0	4741.2
5'-Flu-d(CGCAT-T-T-Py-ATCGC)	10.3	4624.0	4624.2
5'-Flu-d(CGCAT-^{Br}U-Phen-T-ATCGC)	9.4	4555.0	4555.0
5'-Flu-d(CGCAT-^{Br}U-Phen-A-ATCGC)	6.3	4563.0	4564.0
5'-Flu-d(CGCAT- T-Phen-A-ATCGC)	7.2	4499.0	4499.1
5'-d(GCGAT-A-A-A-ATGCG)	14.7	4008.0	4007.6
5'-d(GCGAT-A-Phen-A-ATGCG)	12.0	4050.0	4050.8
5'-d(GCGAT-Py-Phen-A-ATGCG)	13.1	4228.0	4227.9

(a) after purification as described above

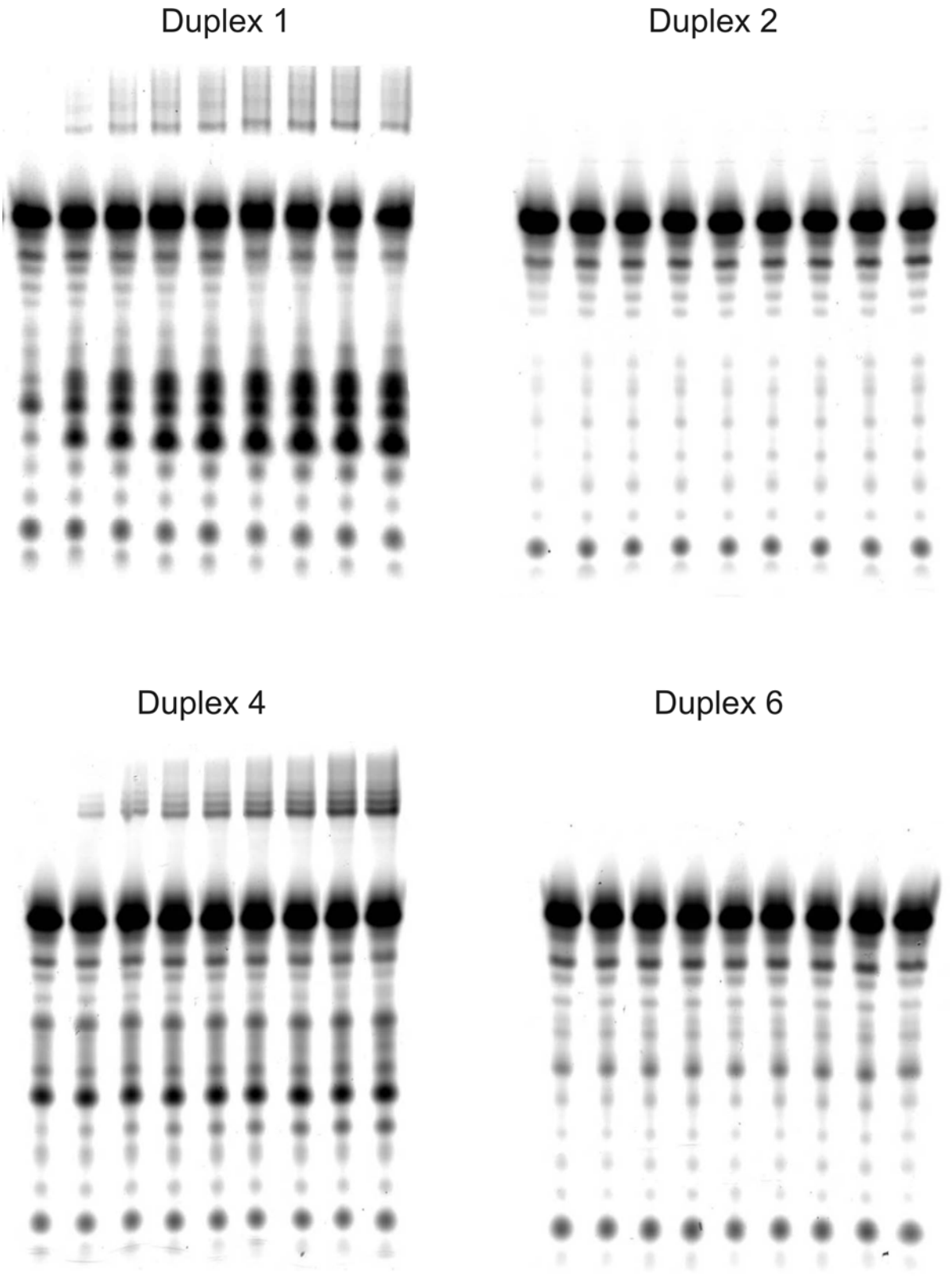
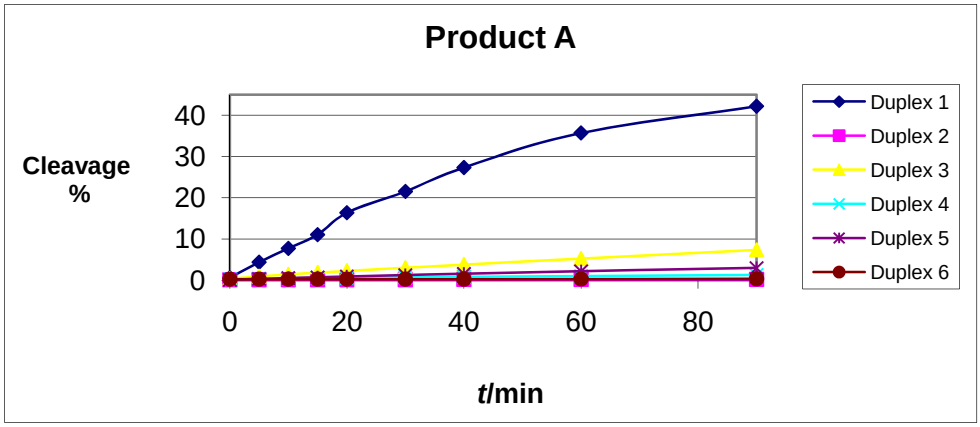
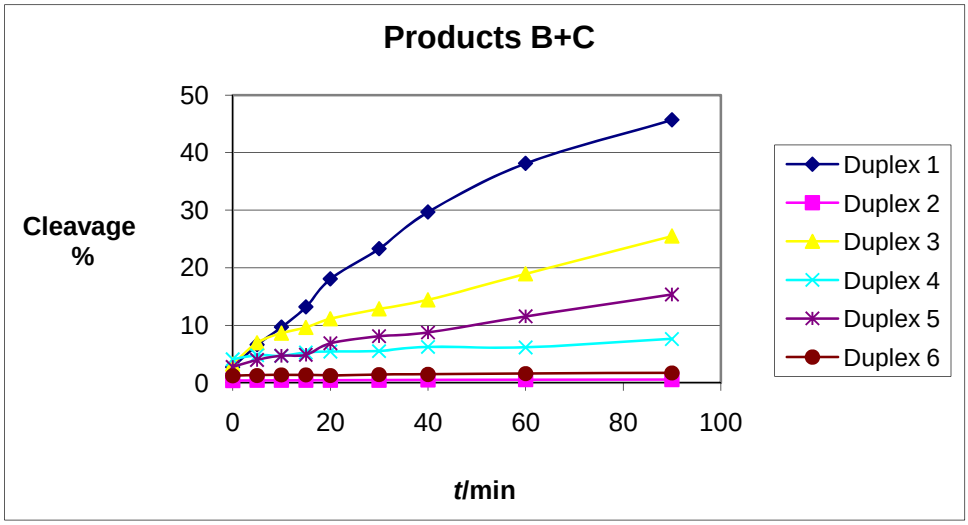


Figure S1. Fluorescence images of 20% denaturing PAGEs showing strand scission of DNA after UV irradiation of duplexes **1, 2, 4** and **6**. Aliquots were withdrawn after 0, 5, 10, 15, 20, 30, 40, 60, 90 min of irradiation and treated with 10% piperidine at 90°C for 30 min.





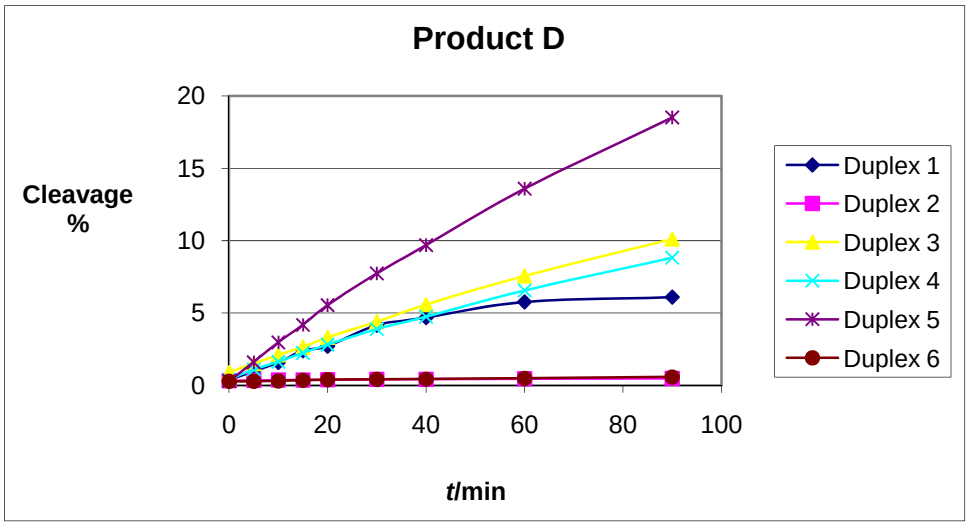


Figure S2. Time evolution of products **a, b+c** and **d** vs. starting material for all duplexes **1-6**.

Results are presented as mean values of two independent experiments.

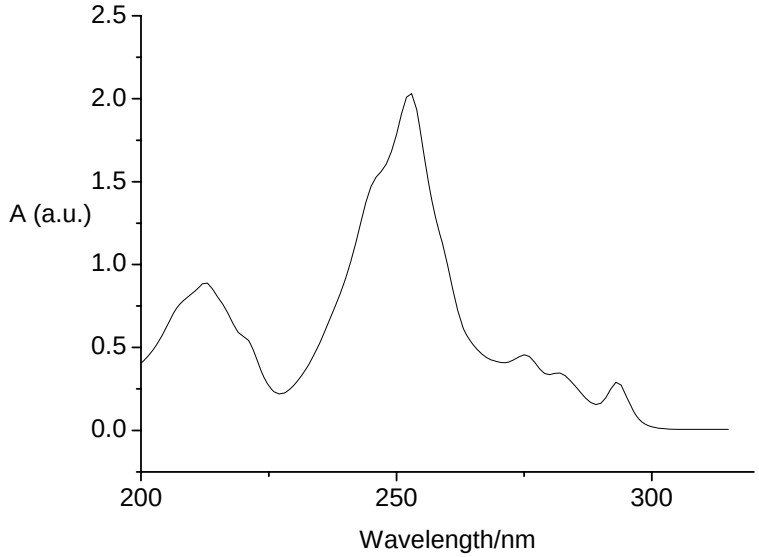


Figure S3. Qualitative UV/VIS-spectrum of the free nucleoside dphen in MeOH.

References for supporting information

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