Electronic Supplementary Information

Enhancing Excess Electron Transport in DNA

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General methods. NMR spectra were recorded on a Bruker AM400, AM500, or AM600 spectrometer and referenced to residual protons in the deuterated solvents. Chemical shifts (δ) and coupling constants (*J*) are reported in parts per million (ppm) and Hertz (Hz), respectively. Mass spectrometry analysis was performed on a JEOL AccuToF-CS ESI-MS in the ESI^+ ionization mode and a Shimadzu Axima-CFR MALDI-TOF. HPLC purification of oligonucleotide conjugates employed a Jasco PU-980 and a reverse phase, Varian C18 Microsorb column. Photoirradiation was performed using a high-pressure Xe-arc (1000 W, Spectral Energy Co.) and a cutoff glass filter (335 nm, WG335, Schott).

General materials. All chemicals, reagents, and solvents of the highest commercial grade were used without further purification unless otherwise noted. All aqueous solutions were prepared with water purified to a resistivity of 17.8-18.0 MΩ.cm. Oligodeoxynucleotides were purchased from TriLink Biotechnologies (^{Br}dU-containing DNA) and Integrated DNA Technologies. All ^{Br}dU-containing DNA was purified by denaturing gel electrophoresis before use. DNA concentration was calculated from its absorption at 260 nm and its ε_{260} value provided by the vender.

Synthesis of *N*-(4-bromobutyloxy)phthalimide (1). Sodium carbonate (1.27 g, 12.0 mmol) and 1,4-dibromobutane (2.57 g, 12.0 mmol) were mixed with acetone (6 ml) in a round bottom flask and heated to reflux under a N_2 atmosphere. Hydroxyphthalimide (489 mg, 3.00 mmol) in acetone (30 ml) was then added dropwise over an hour. The reaction was refluxed for 20 h and then cooled and filtered. The filtrate was concentrated under reduced pressure. The desired product was isolated after silica gel flash chromatography (hexanes:ethyl acetate, 1:0 to 1:1) as a white powder in 95% yield (850 mg, 2.85 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (m, 2H) 7.76 (m, 2H), 4.24 (t, J = 12.2 Hz, 2H), 3.54 (t, J = 13.1 Hz, 2H), 2.15 (m, 2H), 1.94 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.1, 136.1, 130.1, 124.5, 78.6, 35.1, 30.4, 28.1. HRMS *m*/*z* calcd for $C_{12}H_{12}BrNO_2$ 298.0065 found 298.0045 ($M + H^+$); mp 68-70 °C.

Synthesis of 2a by alkylation of 1,5-diaminonaphthalene with *N*-(4-bromobutyloxy)-phthalimide. The bromide **1** (298 mg, 1.00 mmol) in acetonitrile (10 ml) was added dropwise to a round bottom flask containing a mixture of 1,5-diaminonaphthalene (632 mg, 4.00 mmol), $NaHCO_3$ (420 mg, 5.00 mmol) and KI (83 mg, 0.50 mmol) in acetonitrile (10 ml). This mixture was refluxed under a N_2 atmosphere for 24 h. After cooling the reaction, the solvent was evaporated. The remaining dark red residue was suspended in water (10 ml), adjusted to pH 5 with 1 N HCl and extracted with dichloromethane (3 × 10 ml). The organic phases were combined, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The desired product **2a** was isolated after silica gel flash chromatography (hexanes:ethyl acetate, 4:1 to 2:3) as an orange solid in 65% yield (244 mg). ^{1}H NMR (500 MHz, CD_3CN) δ 7.60 (dd, J = 19.5, 7.4 Hz, 2H), 7.50 (dd, J = 9.5 Hz, 2H), 7.31 (t, J = 8.0 Hz, 2H), 7.25 (t, J = 7.9 Hz, 2H) 6.97 (d, J = 9.5 Hz, 2H), 6.76 (d, J = 7.3 Hz, 2H), 4.50 (s, 3H), 3.24 (m, 4H), 1.94 (m, 4H). ^{13}C NMR (500 MHz, CD_3CN) δ 165.1, 149.3, 145.0, 130.5, 126.3, 126.1, 125.9, 118.7, 115.9, 115.7, 113.1, 110.0, 78.6, 53.7, 31.4, 25.7. HRMS *m*/*z* calcd for $C_{22}H_{21}N_3O_3$ calcd 376.1616 found 376.1612 ($M + H^+$); mp 122-124 °C.

Synthesis of 3a by deprotection of *N*-(4-oxyphthalimidebutyl)-1,5-diaminonaphthalene. Hydrazine monohydrate (0.70 g, 14 mmol) was added to a solution of **2a** (19 mg, 0.051 mmol) in EtOH:CH_2Cl_2 (1:1, 3 ml), and the mixture was stirred at room temperature for 25 min. Dichloromethane (4 ml) and sulfuric acid (2%, 8 ml) were added to the mixture, and it was filtered through celite 545® following a published protocol.[1] The filter cake was then washed successively with dichloromethane (2 ml) and 2% sulfuric acid (8 ml). The aqueous phase was washed with dichloromethane (2 × 5 ml). The combined organic phase was dried over $MgSO_4$, filtered and evaporated under reduced pressure to yield an orange solid **3a** in a quantitative yield (12 mg). ^{1}H NMR (500 MHz, CD_2Cl_2) δ 7.31 (m, 1H), 7.24 – 7.18 (m, 2H), 7.18 – 7.13 (dd, 1H), 6.76 – 6.72 (m, 1H), 6.61-6.58 (d, J = 7.6 Hz, 1H), 4.61 – 3.86 (s, 3H), 3.72 (m, 2H), 3.28 (m, 2H), 1.73-1.82 (m, 4H). ^{13}C NMR (126 MHz, CD_2Cl_2) δ 144.7, 143.6, 126.1, 125.6, 124.8, 124.7, 110.8, 110.1, 110.0, 104.7, 76.2, 44.7, 26.9, 26.6. HRMS *m/z* calcd for $C_{14}H_{19}N_3O$ calcd 246.1558 found 246.1560 ($M + H^+$).

Synthesis of 2b by alkylation of 1-aminoanthracene with *N*-(4-bromobutyloxy)phthalimide. The bromide **1** (298 mg, 1.00 mmol) in acetonitrile (10 ml) was added dropwise to a round bottom flask containing a mixture of 1-aminoanthracene (194 mg, 1.05 mmol), $NaHCO_3$ (169 mg, 2.01 mmol) and KI (83 mg, 0.50 mmol) in acetonitrile (5 ml). This mixture was refluxed under a N_2 atmosphere for 24 h. After the reaction was cooled, the solvent was evaporated. The residue was suspended in water (10 ml), adjusted to pH 5 by addition of 1 N HCl and was extracted with dichloromethane (3 × 10 ml). The organic phases were combined, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The desired product **2b** was isolated from silica gel flash chromatography (hexanes:ethyl acetate, 1:0 to 3:2) as a brown solid in 65% yield (267 mg, 0.65 mmol). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.5 (s, 1H), 8.3 (s, 1H), 7.9-8.1 (m, 2H), 7.7-7.9 (m, 4H), 7.2-7.5 (m, 4H), 6.5-6.6 (m, 1H), 4.7 (s, 1H), 4.3 (m, 2H), 3.4 (m, 2H), 1.9-2.2 (m, 4H). ^{13}C NMR (400 MHz, $CDCl_3$) 163.8, 141.7, 134.5, 132.6, 131.6, 128.9, 128.5, 127.9, 126.6, 125.8, 125.4, 124.9, 123.5, 119.3, 118.8, 117.7, 107.6, 102.4, 78.2, 44.1, 26.3, 25.6. HRMS *m/z* calcd for $C_{26}H_{22}N_2O_3$ 411.1663 found 411.1704 ($M + H^+$).

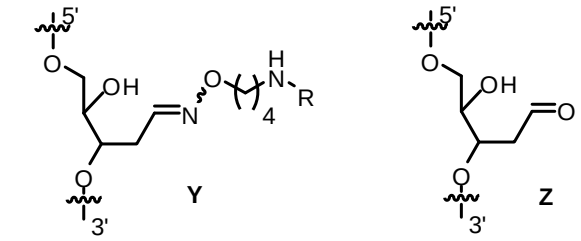
Synthesis of 3b by deprotection of *N*-(4-oxyphthalimidebutyl)-1-aminoanthracene. Hydrazine monohydrate (0.70 g, 14.0 mmol) was added to a solution of **2b** (25 mg, 0.063 mmol) in EtOH:CH_2Cl_2 (1:1, 3 ml), and the mixture was stirred at room temperature for 40 min. Dichloromethane (4 ml) and sulfuric acid (2%, 8 ml) were added to the mixture, and it was filtered through celite 545® following a published protocol.[1] The filter cake was then washed successively with dichloromethane (2 ml) and 2% sulfuric acid (8 ml). The aqueous phase was washed with dichloromethane (2 × 5 ml). The combined organic phase was dried over $MgSO_4$, filtered and evaporated under reduced pressure to yield an orange solid **3b** in a quantitative yield (17 mg). ^{1}H NMR (600 MHz, CD_3OD) δ 7.7-8.0 (m, 4H), 7.4 (m, 2H), 7.2 (m, 2H), 6.5 (m, 1H), 4.6 (s, 1H), 3.7 (m, 2H), 3.3 (m, 2H), 1.7-2.0 (m, 4H). ^{13}C NMR (500 MHz, CD_2Cl_2) δ 143.0, 133.4, 132.5, 131.8, 129.2, 128.6, 127.2, 126.4, 126.0, 124.5, 124.2, 120.4, 119.6, 108.0, 73.6, 44.9, 28.0, 26.7. HRMS *m/z* calcd for $C_{18}H_{20}N_2O$ 281.1607 found 281.1590.

Conjugation of electron donors, 3a and 3b, to DNA. Oligonucleotides containing internal abasic sites were generated from uridine containing analogues **OD7'** and **OD8'** (see **Chart S1**) using uracil-DNA deglycosylase as described previously.[2] Oligonucleotides containing terminal abasic sites (**OD5'** and OD**6')** were purchased from Trilink Biotechnologies. Solutions of **3a** and **3b** (50 μL, 50 mM in HPLC grade acetonitrile) were alternatively combined with oligonucleotides containing an abasic DNA (20 μL, 1 mM in water) and incubated at 37 ºC for 1 h. Excess donor was removed with a BioSpin 6 column (prewashed with water, 3 times), and the conjugates were isolated from reverse phase (C-18) HPLC using a gradient of 10% acetonitrile in 45 mM triethylammonium acetate (pH 6.0) to 30% acetonitrile in 35 mM trietyl-ammonium acetate (pH 6.0) over 20 min (1 ml/min) in yields about 30% (based on A_{260} of starting material) (See for example, **Figure S1**). MALDI (with a matrix of 3,5-dimethoxy-4-hydroxycinnamic acid) *m/z* calcd for **OD1'a** and **OD3'a** $C_{185}H_{236}N_{65}O_{107}P_{17}$ 5608.8, found 5608.3 and 5609.2, calcd for **OD1'b** and **OD3'b** $C_{189}H_{237}N_{64}O_{107}P_{17}$ 5643.4 found 5643.2 and 5644.1, calcd for **OD2'a** and **OD4'a** $C_{185}H_{236}N_{65}O_{106}P_{17}$

5592.8 found 5591.7 and 5592.4, calcd for **OD2'b** and **OD4'b** $C_{189}H_{237}N_{64}O_{106}P_{17}$ 5627.4 found 5627.9 and 5627.8, respectively.

Photochemical initiation of EET in DNA. DNA strands containing ^{Br}dU were radiolabeled at the 5'-terminus using [γ-^{32}P]ATP (Amersham Bioscience) and T4 polynucleotide kinase (New England Biolabs) following standard procedures. The radiolabeled strand (0.2 μM) and the complementary conjugate strand (1.1 – 1.6 equivalents) was annealed in 10 mM sodium phosphate pH 7 and 100 mM NaCl by heating to 90 °C followed by slow cooling to room temperature. Aliquots of this solution (10 μL each) were then irradiated in microcentrifuge tubes under aerobic conditions (10 °C). DNA was precipitated with sodium acetate (3 M, pH 5.5, 5 µL) and ethanol (200 µL), treated with 10% piperidine (15 µL) at 90 °C for 30 min, and dried under reduced pressure. The resulting residue was resuspended in loading buffer (DMF, 0.1% bromphenol blue, 0.1% xylene cyanol FF) and analyzed by electrophoresis using a 20% denaturing polyacrylamide gel (acrylamidebisacrylamide 19:1, 7 M urea). Strand scission was quantified and reported relative to the total material in each lane as measured by phosphorimagery and its software ImageQuant (GE Healthcare Life Sciences).

OD1' 5'-CGT CAT G**Y**A ATG TAC TGC
OD2' 5'-CGT CAT A**Y**A ATG TAC TGC
OD3' 5'-AAT GTA CTG CCG TCA TG**Y**
OD4' 5'-AAT GTA CTG CCG TCA TA**Y**
OD5' 5'-AAT GTA CTG CCG TCA TG**Z**
OD6' 5'-AAT GTA CTG CCG TCA TA**Z**
OD7' 5'-CGT CAT GUA ATG TAC TGC
OD8' 5'-CGT CAT AUA ATG TAC TGC



R = a, 5-naphthalene;
b, 1-anthracene

Chart S1. Nucleotide sequences of oligonucleotides and their conjugates.

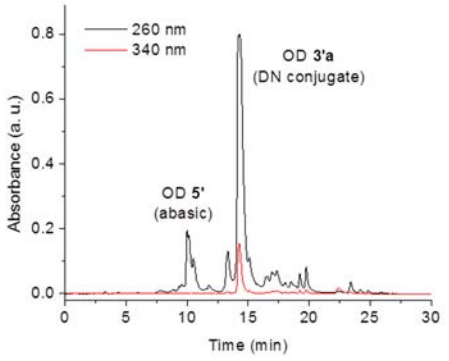


Figure S1. A sample separation of an oligonucleotide containing an abasic site (**OD5'**) and its DN conjugate (**OD3'a**) by reverse phase (C-18) HPLC using a gradient of 10% acetonitrile in 45 mM triethylamine acetate buffer (pH 6.0) to 30% acetonitrile in 35 mM trietylamonium acetate (pH 6.0) over 20 min (1 ml/min).

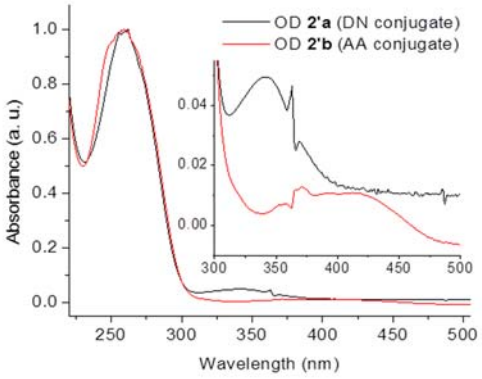


Figure S2. Absorption spectra of DN- and AA-containing oligonucleotide conjugates. A change in lamps within the spectrophotometer is evident at ca. 375 nm. The integrated area under the absorption traces above 330 nm for DN:AA was 1.0:0.9.

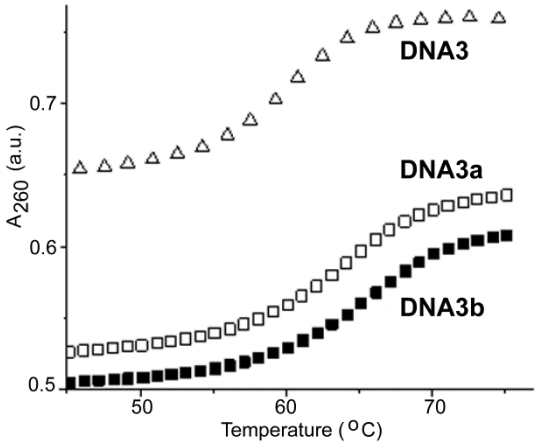


Figure S3. Sample melting of DNA duplexes (△) parent **DNA3** containing an abasic site, (□) **DNA3a** containing a conjugated DN and (■) **DNA3b** containing a conjugated AA. DNA samples were pre-annealed in 50 mM sodium phosphate pH 7 and 50 mM NaCl.

	T_m (°C)		
Duplex	abasic parent	DN conjugate	AA conjugate
DNA1	61	65	67
DNA2	60	65	66
DNA3	59	64	65
DNA4	58	64	65

Table S1. Melting temperatures for DNA duplexes containing abasic sites and their DN- and AA-conjugates under conditions described in Figure S3. A_{260} was monitored with a Varian Cary 100 UV-Vis spectrophotometer while heating samples (0.5 °C/min) T_m values were defined by the midpoint transition.

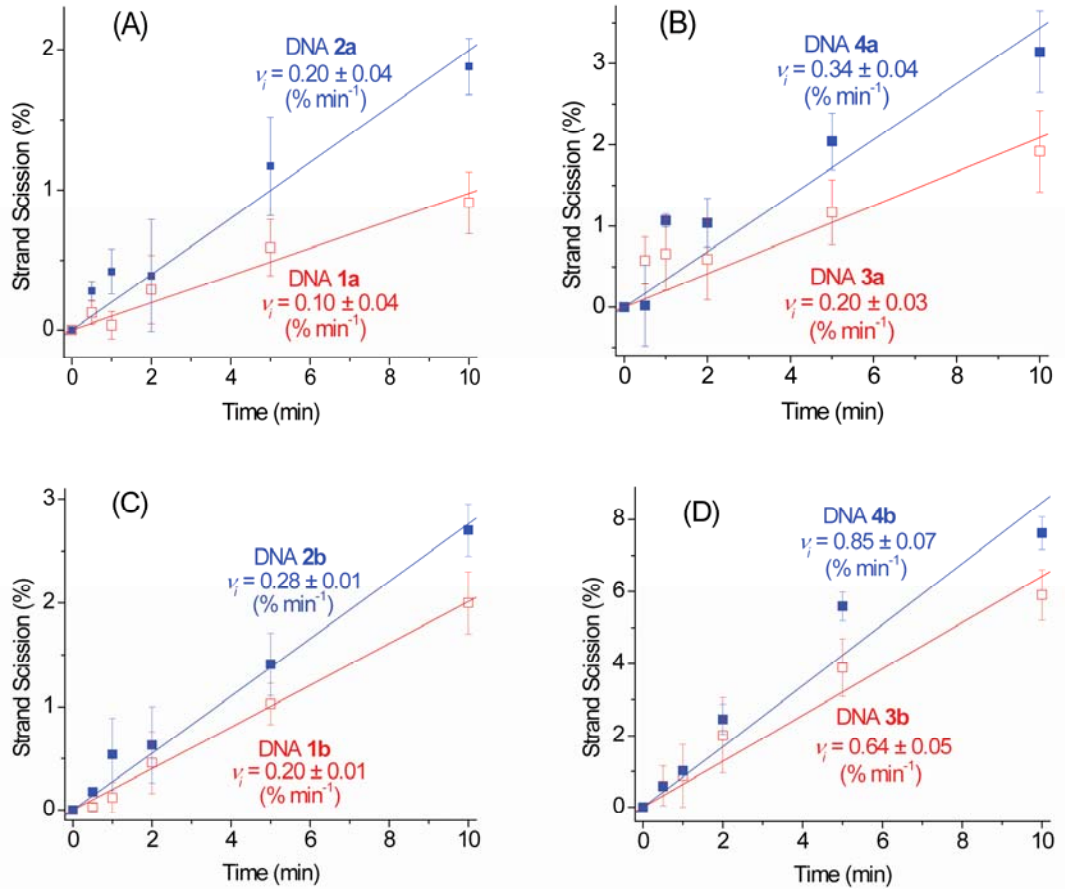


Figure S4. Initial rates of strand scission by photoinduced EET from donor (DN and AA) to acceptor (^{Br}dU) in duplex DNA. Red unfilled and blue filled squares correspond to data observed from the internal and terminal conjugates, respectively. Duplex DNA (0.2 μM, 90 nCi, ^{Br}dU strand) in sodium phosphate (10 mM, pH 7.0) and NaCl (100 mM) were irradiated (λ > 335 nm) for the indicated times. Initial rates were calculated from the linear fit of strand scission (% vs. total) resulting from electron capture by ^{Br}dU (See **Figure S5**). Each experiment was repeated at least three times and error bars represent standard deviations.

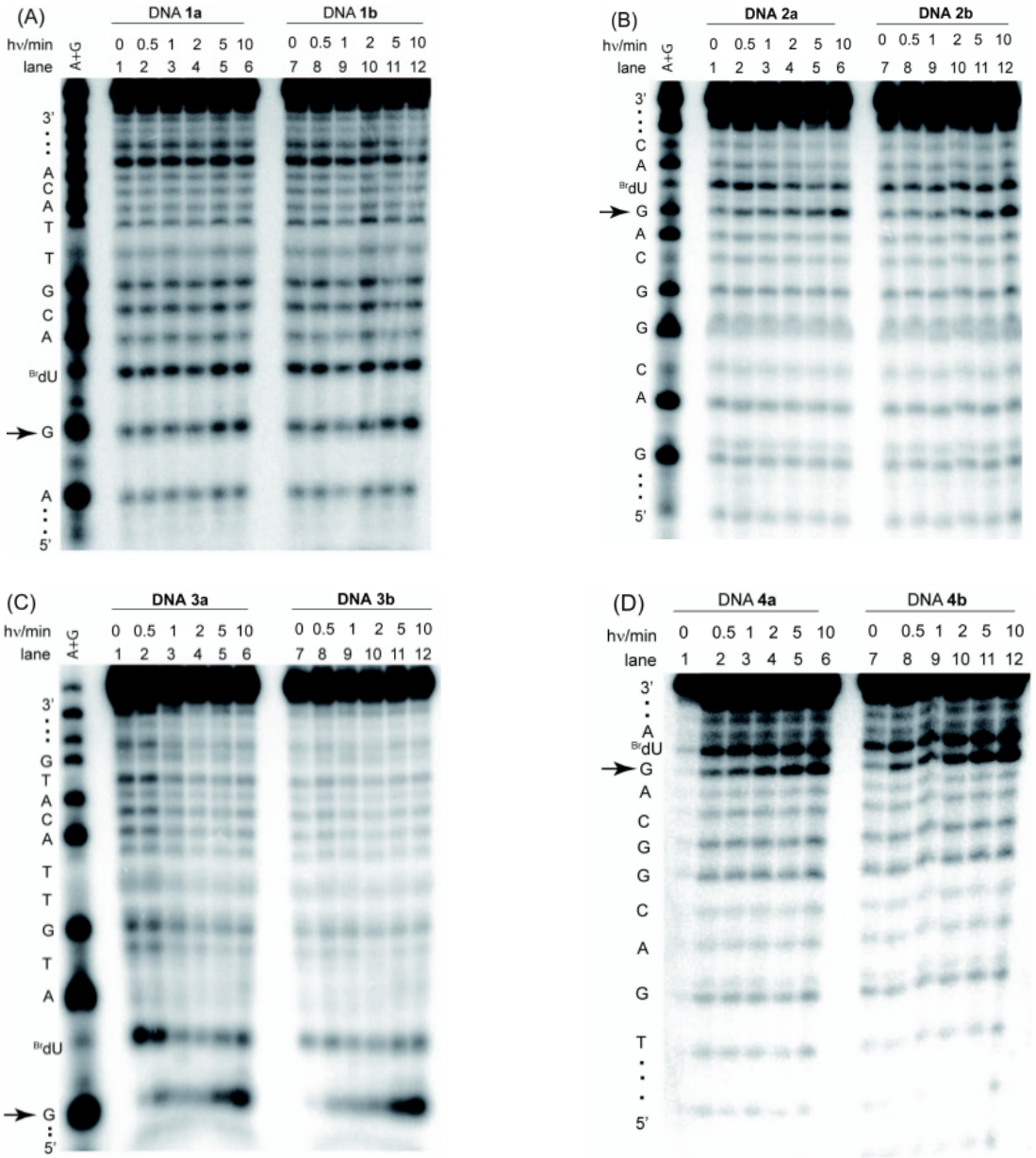


Figure S5. Phosphoimagery of 20% denaturing polyacrylamide gels of DNA after UV irradiation (> 335 nm, 10 ºC) and subsequent treatment with 10% piperidine at 90 ºC for 30 min. (A) DN- and AA-conjugates **DNA1a**,**b** (B) DN- and AA-conjugates **DNA2a,b**, (C) DN- and AA-conjugates **DNA3a,b** and (D) DN- and AA-conjugates **DNA4a,b**. In each study, the ^{Br}dU containing strand was radiolabeled at the 5'-terminus with γ-^{32}P-ATP. Lanes 1-6 correspond to DNA with conjugated DN and lanes 7-12 correspond to DNA with conjugated AA as the electron donor. Arrows indicate the scission site based on electron capture by ^{Br}dU.

References:

1) S. K. Jackson, A. Karadeolian, A. B. Driega and M. A. Kerr, Stereodivergent methodology for the synthesis of complex pyrrolidines, *J. Am. Chem. Soc.*, 2008, **130**, 4196-4201.

2) A. Fakhari M. and S. E. Rokita, A new solvatochromic fluorophore for exploring nonpolar environments created by biopolymers, *Chem. Commun.*, 2011, **47**, 4222-4224.