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 (BrdU-containing DNA) and Integrated DNA Technologies. All BrdU-containing DNA was purified by denaturing gel electrophoresis before use. DNA concentration was calculated from its absorption at 260 nm and its ε260 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 N2 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). 1H NMR (400 MHz, CDCl3) 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). 13C NMR (126 MHz, CDCl3) δ 165.1, 136.1, 130.1, 124.5, 78.6, 35.1, 30.4, 28.1. HRMS m/z calcd for C12H12BrNO2 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), NaHCO3 (420 mg, 5.00 mmol) and KI (83 mg, 0.50 mmol) in acetonitrile (10 ml). This mixture was refluxed under a N2 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 MgSO4, 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). 1H NMR (500 MHz, CD3CN) 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). 13C NMR (500 MHz, CD3CN) 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 C22H12BrNO2 376.1616 found 376.0045 (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:CH2Cl2 (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. 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 MgSO4, filtered and evaporated under reduced pressure to yield an orange solid 3a in a quantitative yield (12 mg). \(^1\)H NMR (500 MHz, CD2Cl2) \(\delta\) 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, CD2Cl2) \(\delta\) 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, 25.6. HRMS \(m/z\) calcd for C189H237N64O107P17 5643.4 found 5643.2 and 5644.1, calcd for C185H236N65O107P17 5608.8, found 5608.3 and 5609.2, calcd for C183H235N65O107P17 5573.7 found 5573.4 and 5574.4.

Synthesis of 2b by alkylation of 1-aminanthracene 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-aminanthracene (194 mg, 1.05 mmol), NaHCO3 (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 MgSO4, 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, CD2Cl2) \(\delta\) 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, CDCl3) \(\delta\) 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 C26H22N2O3 411.1663 found 411.1704 (M + H\(^+\)).

Synthesis of 3b by deprotection of \(N\)-(4-oxyphthalimidebutyl)-1-aminanthracene.  
Hydrazine monohydrate (0.70 g, 14.0 mmol) was added to a solution of 2b (25 mg, 0.063 mmol) in EtOH:CH2Cl2 (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\(^\circ\) following a published protocol. 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 MgSO4, filtered and evaporated under reduced pressure to yield an orange solid 3b in a quantitative yield (17 mg). \(^1\)H NMR (600 MHz, CD3OD) \(\delta\) 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 (600 MHz, CD3OD) \(\delta\) 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 C18H20N2O 281.1607 found 281.1590 (M + H\(^+\)).

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. Oligonucleotides containing terminal abasic sites (OD5' and OD6') were purchased from Trilink Biotechnologies. Solutions 3a and OD8' (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 °C 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 triethylammonium acetate (pH 6.0) over 20 min (1 ml/min) in yields about 30% (based on A260 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 C183H236N65O107P17 5608.8, found 5608.3 and 5609.2, calcd for OD1’b and OD3’b C189H237N64O107P17 5643.4 found 5643.2 and 5644.1, calcd for OD2’a and OD4’a C185H236N65O106P17 5573.7 found 5573.4 and 5574.4.
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-32P]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 (acrylamide: bisacrylamide 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).

**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 triethylammonium 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.

**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.

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<tr>
<th>Duplex</th>
<th>$T_m$ (°C)</th>
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<tbody>
<tr>
<td></td>
<td>abasic parent</td>
</tr>
<tr>
<td>DNA1</td>
<td>61</td>
</tr>
<tr>
<td>DNA2</td>
<td>60</td>
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<tr>
<td>DNA3</td>
<td>59</td>
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<tr>
<td>DNA4</td>
<td>58</td>
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**Figure S4.** Initial rates of strand scission by photoinduced EET from donor (DN and AA) to acceptor (BrdU) 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, BrdU strand) in sodium phosphate (10 mM, pH 7.0) and NaCl (100 mM) were irradiated ($\lambda > 335$ nm) for the indicated times. Initial rates were calculated from the linear fit of strand scission (% vs. total) resulting from electron capture by BrdU (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 °C) and subsequent treatment with 10% piperidine at 90 °C 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 BrdU containing strand was radiolabeled at the 5’-terminus with γ-32P-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 BrdU.

References: