Real-Time Spectroscopic and Chemical Probing of
Reducive Electron Transfer in DNA**

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Materials and Methods. MALDI-TOF analysis 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 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, 10 mM Na-P_i-buffer, 260 nm, 10-80°C, interval 1°C, scan speed 0.5°C / min) were recorded on a Varian Cary Bio 100 spectrometer. The absorption spectra (15.6 µM duplex) were recorded on a Varian Cary 50 spectrometer, the fluorescence spectra (15.6 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 an emission and are intensity corrected.

Preparation and Characterization of Oligonucleotides (General Procedure). The oligonucleotides were prepared on an Expedite 8909 DNA synthesizer (Applied Biosystems) via standard phosphoramidite protocols using CPGs (1 µmol) and chemicals from ABI and Glen Research. After preparation, the trityl-off oligonucleotide was cleaved off the resin and was deprotected by treatment with conc. NH₄OH at 55°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=NH₄OAc buffer (50 mM), pH=6.5; B= MeCN; gradient=0-15% B over 45 min. The oligonucleotides were lyophilised 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 pyrene-
modified oligonucleotides in the presence of 1 equiv. unmodified complementary strand to 80 °C, followed by slow cooling.

**Preparation and Characterization of Py-dU- and BrdU-Modified Oligonucleotides.** The oligonucleotides were prepared on an Expedite 8909 DNA synthesizer (Applied Biosystems) via standard phosphoramidite protocols using CPGs (1 µmol) and chemicals from ABI and Glen Research. The Py-dU building block (see: N. Amann, E. Pandurski, T. Fiebig, H.-A. Wagenknecht, *Angew. Chem.* 2002, 114, 3084-3087; *Angew. Chem. Int. Ed.* 2002, 41, 2978-2980.) and the Br-dU building block from ChemGenes coupled nearly quantitatively. After preparation, the trityl-off oligonucleotide was cleaved off the resin and was deprotected by treatment with conc. NH₄OH at rt. for 27 h, protected from light. The oligonucleotide was dried and purified by HPLC on a semipreparative RP-C18 column (300 Å, Supelco) using the following conditions: A=NH₄OAc buffer (50 mM), pH=6.5; B= MeCN; gradient=0-30% B over 45 min. The oligonucleotides were lyophilised 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, using ε (260 nm)=14.600 M⁻¹ cm⁻¹ for Py-dU (see: N. Amann, E. Pandurski, T. Fiebig, H.-A. Wagenknecht, *Angew. Chem. Int. Ed.* 2002, 41, 2978-2980; *Angew. Chem.* 2002, 114, 3084-3087.) and ε (260 nm)=7.000 M⁻¹ cm⁻¹ for Br-dU (product information, ChemGenes).

**Strand Cleaving Experiments.** Duplexes (4 µM DNA, 10 mM Na-Pi-buffer, 250 mM NaCl) are prepared by heating equimolar solutions of the single strands to 80°C for 10 min. in the dark and subsequent slow cooling. The measurements were
performed in quartz glass cuvettes (1 cm). The freshly prepared duplexes were
irradiated by a Xenon lamp (75 W Xe lamp, 14 V, 5.4 A; Oriel Instruments) equipped
with a cut-off filter (Andover Corporation, >305 nm). Every 5 min. aliquots (30 µL) of
the sample solution (1000 µL) were taken to a RNAse/DNAse free container and
stored protected from light at rt. Subsequent to the withdrawal of the last sample after
60 min., piperidine (3 µL) was added to all samples. The samples were heated to
90°C (30 min.), lyophilised, dissolved in water (15 µL) and analysed by HPLC (RP-
C18, Supelco) under the following conditions: A=NH₄OAc buffer (50 mM), pH=6.5; B=
MeCN; gradient=0-30% B over 30 min. The obtained peaks were processed to give a
ratio between unmodified and modified DNA-single strand.

Table S1. MS (MALDI-TOF) data of ss1-ss5.

<table>
<thead>
<tr>
<th>oligonucleotide</th>
<th>m/z (calcd.)</th>
<th>m/z (exp.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ss1</td>
<td>5306</td>
<td>5322</td>
</tr>
<tr>
<td>ss2</td>
<td>5393</td>
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<td>ss3</td>
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<td>ss4</td>
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<td>5423</td>
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<tr>
<td>ss5</td>
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<td>5423</td>
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Table S2. Melting temperatures ($T_m$) of duplexes 1-5.

<table>
<thead>
<tr>
<th>DNA duplex</th>
<th>$T_m$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>57</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
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</tr>
<tr>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
</tr>
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</table>

**Femtosecond Broadband Pump-Probe Setup.** The Py-dU-modified DNA duplexes were exited by pump pulses at 350 nm. The changes in optical density were probed by a femtosecond white-light continuum (WLC) generated by tight focusing of a small fraction of the output of a commercial Ti:Sa based pump laser (CPA-2001, Clark-MXR) into a 3 mm CaF$_2$ plate. The obtained WLC provided a usable probe source between 330 and 750 nm. The WLC was split into two beams (probe and reference) and focused into the sample using reflective optics. After passing through the sample both probe and reference were spectrally dispersed and simultaneously detected on a CCD sensor. The pump pulse (350 nm, 100-200 nJ) was generated by frequency doubling of the compressed output of a commercial NOPA system (Clark-MXR, 680 nm, 8 µJ, 30 fs). To compensate for group velocity dispersion in the UV-pulse we used an additional prism compressor. Independent measurements of the chirp of the WLC were carried out to correct the pump-probe spectra for time-zero differences. The overall time resolution of the setup was obtained from the rise time of the signal (above 580 nm). Assuming a Gaussian shape cross-correlation we obtained a width
of 100-120 fs (FWHM). A spectral resolution of 7-10 nm was obtained. Measurements were performed with magic angle geometry (54.7°) for the polarization of pump and probe pulses to avoid contributions from orientational relaxation. Pump energy and pump spot size (~ 200-400 µm) were adjusted to minimize contributions from the solvent to the signal. Steady state absorption and fluorescence spectra of the samples measured before and after the time resolved experiments were compared with each other and no indications for degradation were found. A sample cell with 1.25 mm fused silica windows and a light path of 1 mm was used for all measurements. The sample concentration was 350 µM DNA duplex.