

Supporting Information

Redmond Red as a Redox Probe for the DNA-mediated Detection of Abasic Sites

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1. Synthesis of modified DNA

All syntheses were performed using standard phosphoramidite chemistry. Thiol-modified DNA strands were synthesized as previously described using a C₆-disulfide phosphoramidite, available from Glen Research. Treatment with excess dithiothreitol in 10 mM Tris buffer (pH 8) for 30 min at ambient temperature resulted in cleavage of the disulfide bond and concomitant removal of the dimethoxytrityl (DMT) protecting group. Nap-5 Sephadex columns (GE Healthcare) were used to isolate the thiolated strands, and reversed-phase HPLC was used to purify the DNA before and after disulfide cleavage (see Figure S1). Redmond Red was incorporated into the DNA sequence at the desired position as a phosphoramidite (also available from Glen Research). Given the sensitivity of Redmond Red to typical cleavage and deprotection conditions (NH₄OH at 60 °C), less harsh conditions were employed (K₂CO₃/MeOH at ambient temperature for 12 – 17 h) for strands containing this modification. Accordingly, phosphoramidites containing more labile protecting groups than standard reagents were required for synthesis (Glen Research). All synthesized DNA strands were purified by reversed-phase HPLC before and after removal of DMT protecting groups using a Varian DYNAMAX C18 semi-preparative column. Isolated products were characterized by MALDI-TOF mass spectrometry, UV-visible spectroscopy, and melting temperature analysis of hybridized duplexes. Representative purification and characterization data are shown below

2. Electrochemical experimental conditions

Prior to self-assembly, gold macroelectrodes (1.6 mm nominal diameter, BAS) were polished on a clean, damp microcloth (Buehler) with alumina slurries of decreasing particle size (1.0 μm, 0.05 μm, Buehler). Electrodes were then etched in H₂SO₄ to expose a fresh, clean surface as well as determine the working area, and rinsed copiously with deionized H₂O. Immediately following, a 20 μl droplet of 50 μM duplex DNA was immobilized on the gold surface and monolayers were allowed to self-assemble overnight at 5 °C. Duplexes were incubated in the presence of 100 mM MgCl₂ in order to encourage the formation of densely packed films. Electrodes were rinsed thoroughly in phosphate buffer (5 mM sodium phosphate, 50 mM NaCl, pH 7) prior to electrochemical measurement.

3. Characterization data

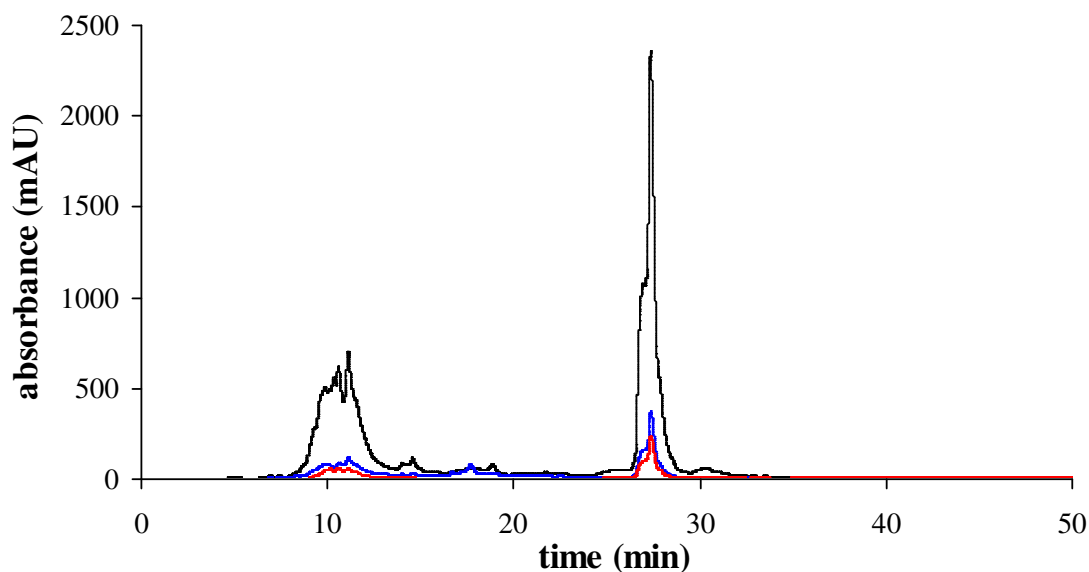


Figure S1a. HPLC chromatogram for Redmond Red-modified DNA (SH- 5' AGT ACT GCA GTA *RR* GCG 3'), prior to disulfide cleavage and DMT deprotection (black: 260nm, blue: 295 nm, red: 580 nm). Failed oligonucleotide syntheses appear around 10 min, while the product elutes around 28 min. Gradient: 100% 50mM NH_4OAc / 0% MeCN to 50% 50 mM NH_4OAc / 50% MeCN in 50 min (1% / min), at 3.5 ml / min.

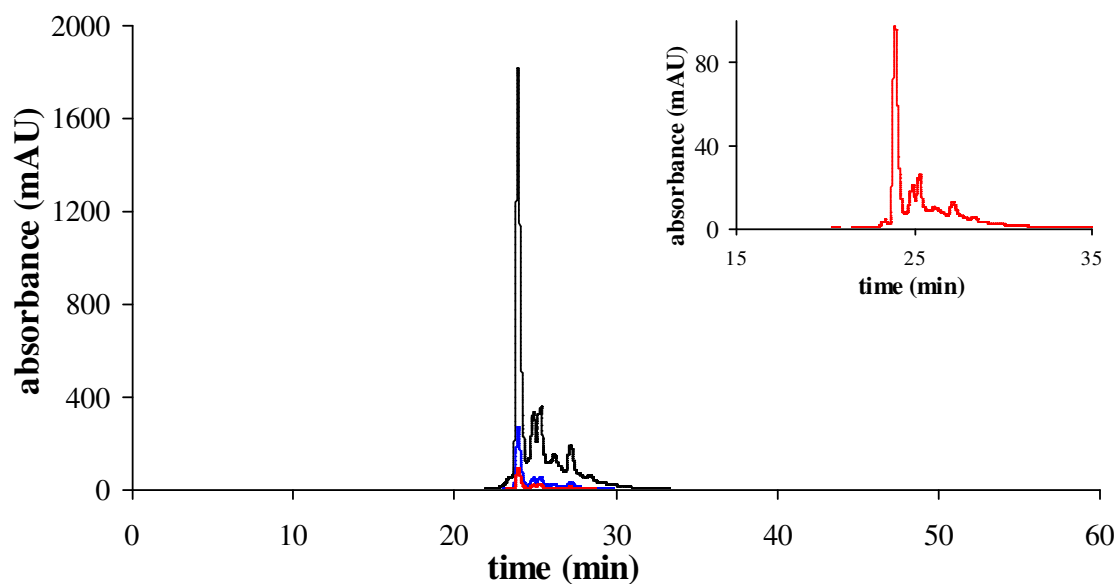


Figure S1b. HPLC chromatogram for Redmond Red-modified DNA, following disulfide cleavage achieved by treatment with DTT (black: 260nm, blue: 295 nm, red: 580 nm). The first main peak, centered around 24 min, corresponds to the product. Mass spectrometry confirmed that the side peaks that follow are not the desired product. Gradient: 100% 50mM NH_4OAc / 0% MeCN to 40% 50 mM NH_4OAc / 60% MeCN in 80 min (0.5% / min), at 3.5 ml / min. Inset: magnified view of the spectrum recorded at 580 nm.

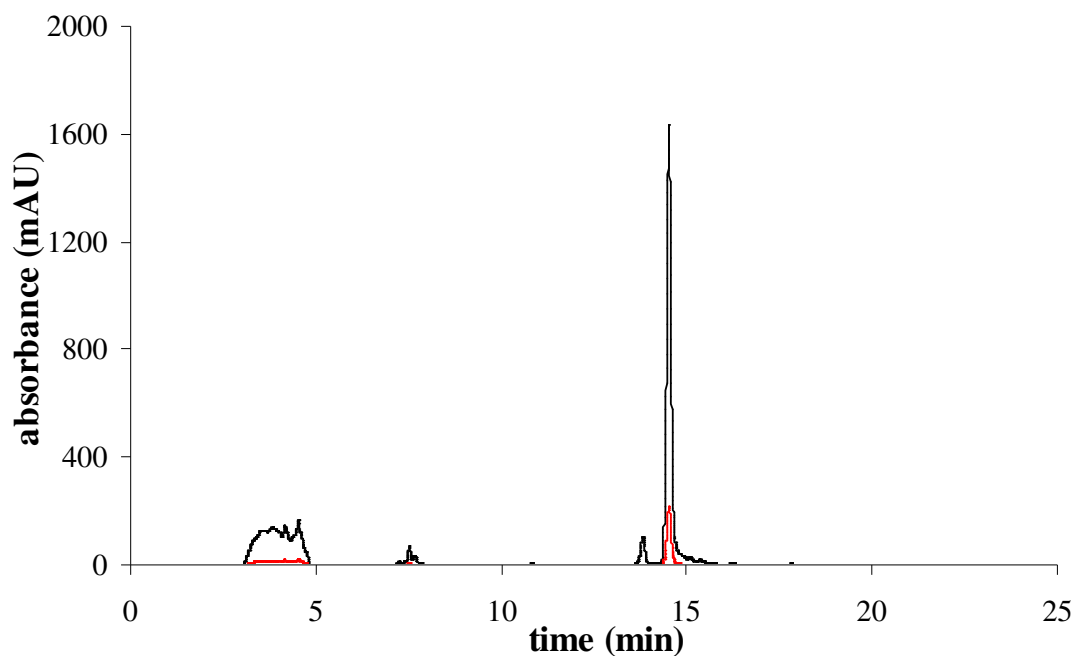


Figure S2a. HPLC chromatogram for DNA strand containing an abasic site (5' CGC __ TAC TGC AGT ACT 3' where "__" indicates the abasic site), prior to removal of DMT protecting group (black: 260nm, red: 294 nm). Product peak appears around 14 min. Gradient: 95% 50mM NH₄OAc / 5% MeCN to 35% 50 mM NH₄OAc / 65% MeCN in 40 min (2% / min), at 3.5 ml / min.

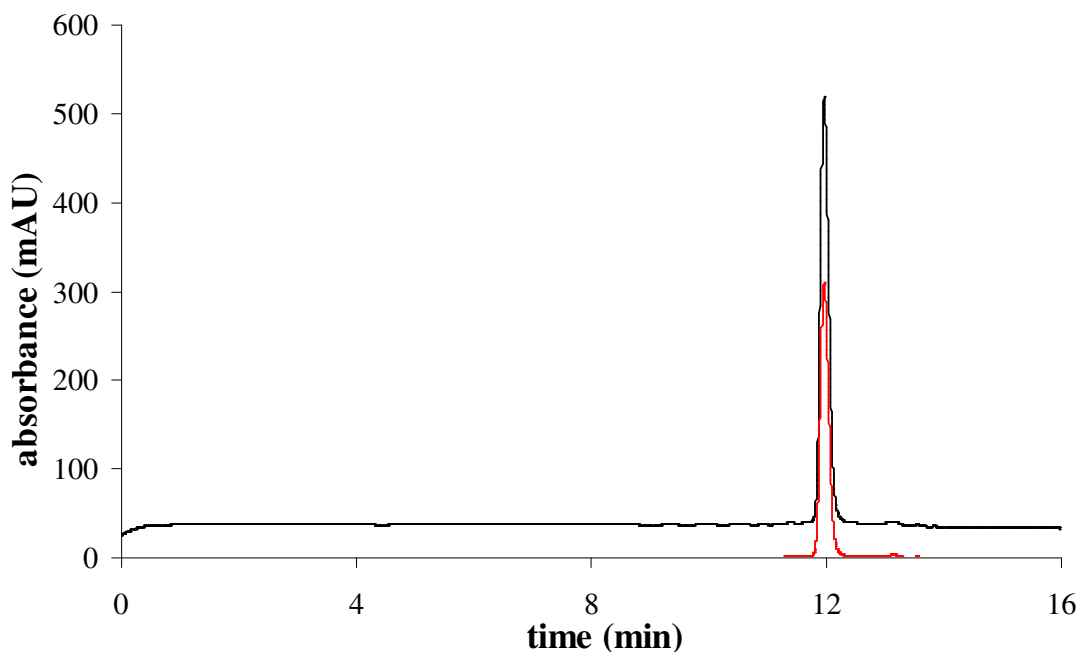


Figure S2b. HPLC chromatogram for DNA strand containing an abasic site, (5' CGC __ TAC TGC AGT ACT 3') following deprotection (black: 260nm, red: 294 nm). Deprotection is achieved by incubating DNA in 80% CH₃HOOH for 30 min and then precipitating with cold EtOH. Gradient: 95% 50mM NH₄OAc / 5% MeCN to 75% 50 mM NH₄OAc / 25% MeCN in 40 min (0.5% / min), at 3.5 ml / min. Product confirmed by mass spectrometry.

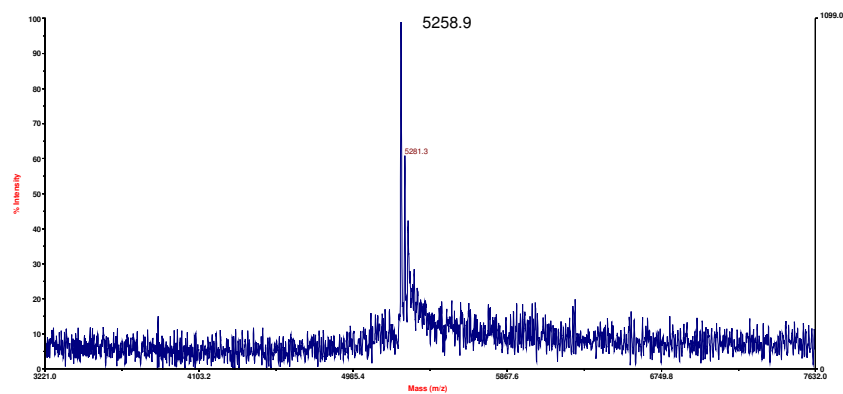


Figure S3a. Mass spectrum of thiolated, Redmond Red modified DNA (^{RR}DNA): SH- 5' AGT ACT GCA GTA RR GCG 3'; $m/z = 5259$ (expected 5254).

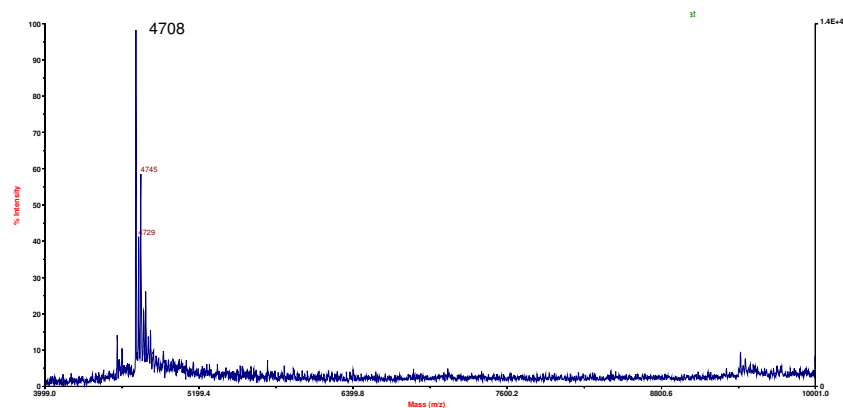


Figure S3b. Mass spectrum of complementary strand containing an abasic site opposite RR: 5' CGC __ TAC TGC AGT ACT 3', where "__" indicates an abasic site; $m/z = 4708$ (expected 4706).

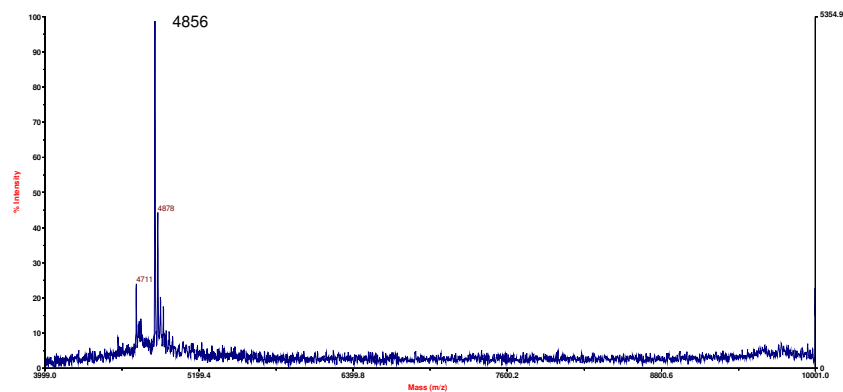


Figure S3c. Mass spectrum of complementary strand containing G opposite RR: 5' CGC G TAC TGC AGT ACT 3'; $m/z = 4856$ (expected 4854).

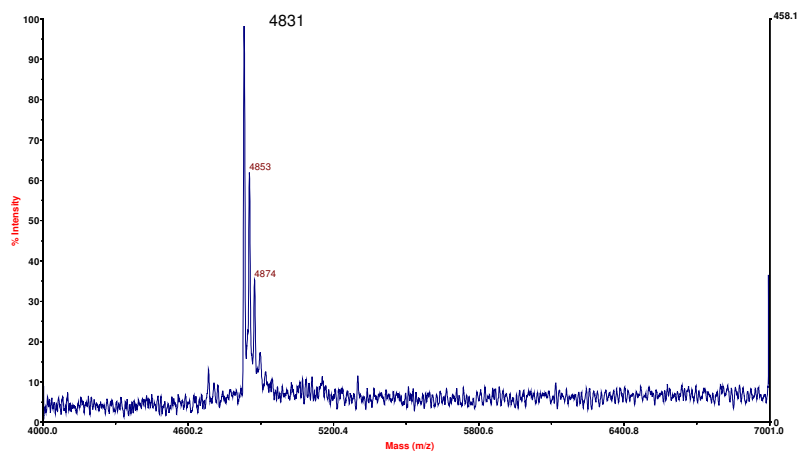


Figure S3d. Mass spectrum of complementary strand containing G opposite RR: 5' CGC *G* TAC TGC AGT ACT 3'; $m/z = 4831$ (expected 4830).

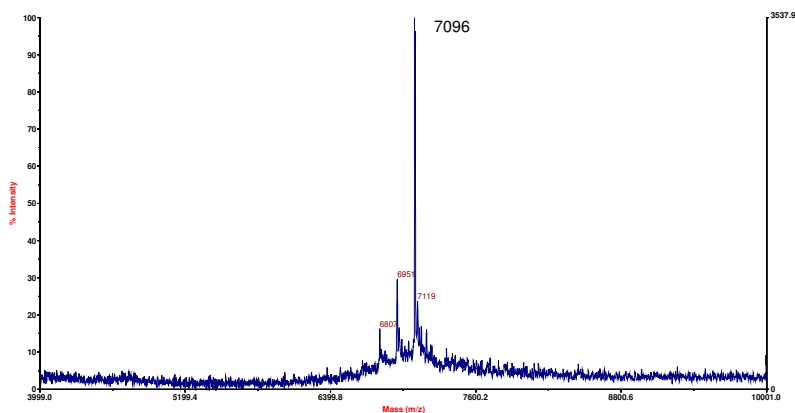


Figure S3e. Mass spectrum of thiolated, Redmond Red modified DNA (^{RR}DNA): SH- 5' AGT ACT GCA GTA *RR* GCG ACT ACG 3'; $m/z = 7096$ (expected 7091).

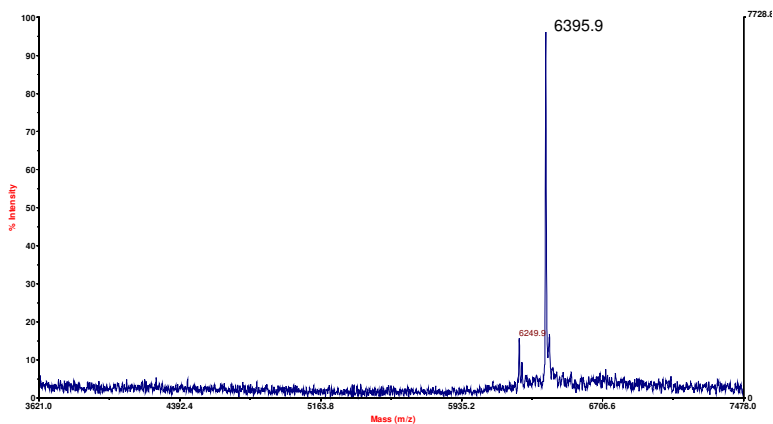


Figure S3f. Mass spectrum of complementary strand containing a 'deletion site' opposite RR: 5' CGT AGT CGC TAC TGC AGT ACT 3'; $m/z = 6396$ (expected 6393).

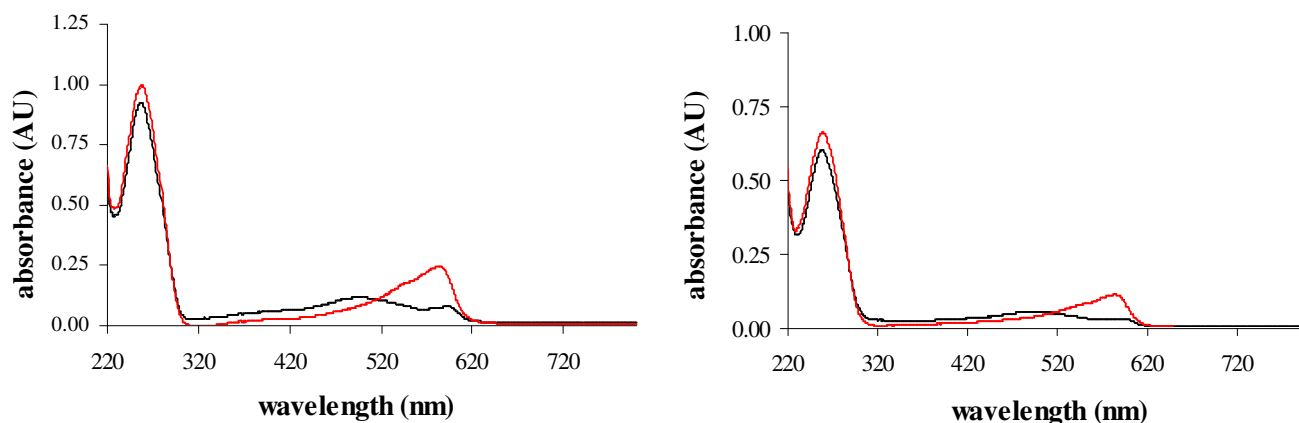


Figure S4. UV-vis spectra of Redmond Red-modified DNA at room temperature (black) and at 80 °C (red), with characteristic maxima at 260 nm (DNA) and 580 nm (RR). At elevated temperatures, the interactions between Redmond Red and the DNA bases are discouraged, thus increasing the absorbance observed at 580 nm. Left: SH – 5' AGT ACT GCA GTA RR GCG 3' (15mer); right: SH – 5' AGT ACT GCA GTA RR GCG ACT ACG 3' (21mer).

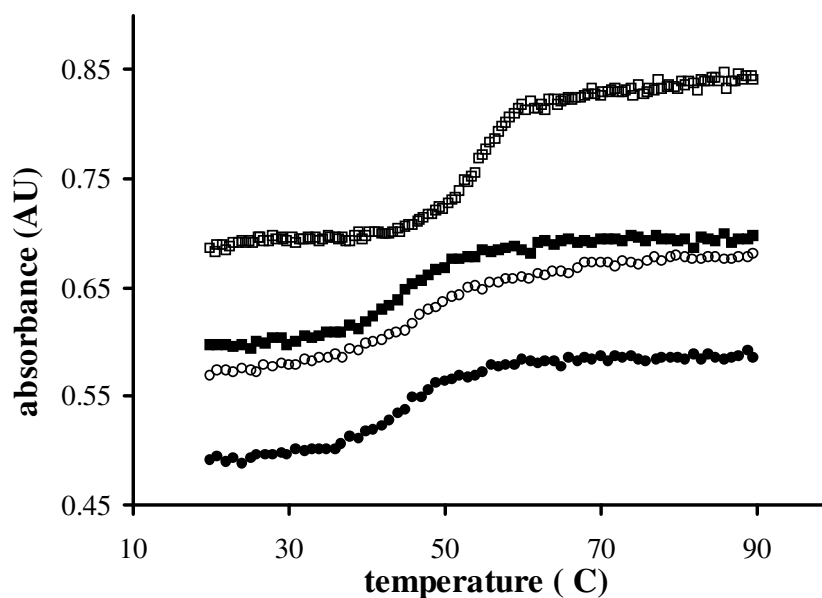


Figure S5. Melting temperature analysis for the four different duplexes employed: Redmond Red opposite a spacer site (closed circles, $T_m = 45$ °C), guanine (open circles, $T_m = 47$ °C), thymine (closed squares, $T_m = 45$ °C) and a deleted site (open squares, $T_m = 54$ °C). For these measurements, duplexes were heated to 90 °C and then cooled back down to 15 °C, at a typical rate of 0.5 °C/min. Absorbance values at 260 nm were recorded at every degree interval.