Supporting Information
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A Twisting Electronic Nanoswitch Made of DNA**
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Supporting Information

Methods

**DNA Purification and Preparation.** All DNA oligonucleotides were purchased from Core DNA Services Inc. (Calgary, AB), and size-purified using denaturing (50% urea, w/v) polyacrylamide gel electrophoresis. The sequences of oligonucleotides used were as follows: “a”: 5’-TGTCA TTATG GCACG TCTGT CTCTG CTGGT GTCGA CTAAA (45 nt). “b”: 5’-GAGCT GCAGA GAGGG GAGTG CCTAT GACTA T (31 nt). “c”: 5’-ATAGT CATAG GCACG TGGGA CACGG TGATC ATAGT T (36 nt). “d”: 5’-AACTA TGATC ACCGT GTGGG GAGTG CCATA ATGAC A (36 nt). A 14-nt oligonucleotide (AQ-P strand) had the sequence: 5’-TTTAG TCGAC CTGA (14 nt). The AQ-P strand was used in several forms. For charge-flow experiments, it had a 5’-amino-modification that was covalently coupled with anthraquinone carboxylic acid NHS ester (AQ-NHS), as described by Huang et al. [1]. Further details of purification are given in the Supporting Information.

The anthraquinone-DNA conjugate was purified by HPLC (Agilent Technologies) as described [1]. For negative control experiments for charge-flow, the 5’-amino-modified AQ-P strand, without conjugation with AQ-NHS, was used. For all other experiments, the unmodified AQ-P strand was used. For the charge-flow experiments, in order to lower the levels of background (i.e. charge-flow unconnected) cleavage, relevant oligonucleotides used were first 5’-end labeled with 32P (using standard kinasing procedures), treated with 10% aqueous piperidine (v/v) at 90 °C for 30 min, followed by lyophilization, and then size-purified by denaturing PAGE.

**Assembly of twDNA:** To assemble twDNA, equal molar concentrations (typically ~4 µM) of oligonucleotides a, b, c, and d were mixed in 10 mM Tris- HCl, pH 7.4, heat-denatured together at 100° C for 3 min, cooled to 40° C, and then made up to Tris buffer (25 mM Tris-HCl, pH 7.4) supplemented to a final concentration of 100 mM LiCl, 100 mM KCl, or 5, 10, or 20 mM of SrCl$_2$ or PbCl$_2$. The solutions were incubated at 37°, for ~12 h. The proper assembly of twDNA (containing the four strands in a
1:1:1:1 stoichiometry) was monitored routinely by one-at-a-time labeling of the individual strands and checking the mobility of the resultant twDNAs against standards (for instance, the partial duplexes formed by two of the strands at a time) by native gel electrophoresis. The DNA solutions were then made up to 1 mM MgCl₂ and hybridized with the short AQ-P strand (see above) for 1 h at 22 °C. The AQ-P strand either had a 5'- appended anthraquinone moiety (for charge transfer experiments) or no such appended moiety (for the methylation protection experiments).

**Anthraquinone-Initiated Charge Transfer through twDNA:** The twDNA (incorporating either an appended AQ, or an unmodified primary amino functionality as a control), dissolved in the appropriate buffer (see above), was irradiated at 0 °C by placing the DNA solutions under a UVP Black-Ray UVL-56 lamp (365 nm) for 1 h, at a distance of 4 cm from the bulb. Constant temperature was maintained with the use of an ice-water bath. Following irradiation, the duplexes were gel-purified in non-denaturing polyacrylamide gels, eluted into TE buffer, ethanol precipitated, and then incubated in 10% piperidine (v/v) at 90 °C for 30 min. Following lyophilization, the DNAs were analyzed in 12% denaturing (50% urea, w/v) polyacrylamide gel electrophoresis.

**Chemical Protection Assays:** Partial DNA modification with dimethyl sulfate (DMS) was carried out using standard procedures. Prior to addition of DMS, twDNA was incubated at 0 °C for 1 h in the appropriate buffered solution (see above). DMS was then added, and the solutions were kept at 30 mins at 22° C, following which unreacted DMS was quenched by the addition of 10% beta-mercaptoethanol. The treated DNA was ethanol precipitated, dried, dissolved in 10% (v/v) piperidine, and heated at 90 °C for 30 min. The DNA was then lyophilized until dry and analyzed by electrophoresis in 12% non-denaturing polyacrylamide gels.

**twDNA switching experiments with Sr²⁺ and EDTA:** twDNA dissolved in Tris buffer containing 10 mM SrCl₂ was incubated for 30 mins at 37° C (“S1” solution).
EDTA was then added to a final concentration of 12.5 mM, and allowed to incubated for 30 mins at 37°C (“E1” solution). The E1 solution was made up to 15 mM SrCl₂, followed by the 37°C incubation, to give the “S2” solution. EDTA was added into the S2 solution to a final concentration of 17.5 mM, to make up solution “E2”. Finally, SrCl₂ to a final concentration of 20mM, followed by the incubation, gave solution “S3”. DNA dissolved in all of the above solutions (S1-S3 and E1-E2) were irradiated for charge transfer, or treated with DMS for structural probing. Following those treatments the DNAs were gel-purified in non-denaturing polyacrylamide gels, eluted into 10 mM Tris, pH 7.4, 1 mM EDTA buffer, ethanol precipitated, and then dissolved an incubated in 10% piperidine (v/v) at 90 °C for 30 min. Following lyophilization, the fragmented DNAs were analyzed by 12% denaturing (50% urea, w/v) polyacrylamide gel electrophoresis.

**Data Analysis:** Imaging and densitometry of sequencing gels were carried out using a Typhoon 9410 Phosphorimager (Amersham Biosciences). Quantitation was facilitated by using Amersham’s ImageQuant 5.2 software. For every quantitation, guanine band intensities from DNAs irradiated for charge flow were corrected for nonspecific cleavage at those same guanines under conditions of non-irradiation.

**Reference**