Supplementary Materials for

Helix-dependent Spin Filtering through the DNA Duplex

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Materials and Methods

DNA Synthesis

All materials for DNA synthesis were purchased from Glen Research. Oligonucleotides were synthesized on an Applied Biosystems 3400 DNA synthesizer using phosphoramidite chemistry on a controlled-pore glass support. The two strands of a duplex were synthesized separately, purified, stored frozen, then annealed prior to electrochemical experiments. The 5'- end of one strand was modified with a C6 S-S phosphoramidite that is later reduced before use. The 5'- end of the complementary strand is either unmodified or modified with an NHS-Carboxy-dT phosphoramidite for later coupling with Nile blue. High pressure liquid chromatography (HPLC) was performed using a reverse-phase PLRP-S column (Agilent) using a gradient of acetonitrile and 50 mM ammonium acetate.

Unmodified DNA. DNA was synthesized using standard phosphoramidites and reagents. After synthesis, the DNA was lyophilized overnight. It was then cleaved from the solid support by incubation at 60^{o} C with concentrated (28-30%) NH_4OH for 12 hours, filtered using CoStar columns, then dried. The dried DNA film was resuspended in phosphate buffer (5 mM phosphate, pH 7, 50 mM NaCl) and HPLC-purified. The DMT (4,4'-dimethoxytrityl) group protecting the 5'- end was then removed by incubation with 80% acetic acid for 45 minutes. The reaction mixture was dried and resuspended in phosphate buffer. The DNA was isolated using HPLC. The purified oligonucleotide was desalted using ethanol precipitation, dried, and the mass was confirmed with Matrix-assisted laser desorption/ionization- time of flight mass spectrometry (MALDI-TOF).

Unmodified oligonucleotides were then stored at -20^{o}C in phosphate buffer until annealing with their complementary strand.

Thiolated DNA. DNA was synthesized using standard phosphoramidites and reagents, with the exception of a C6 S-S phosphoramidite that was attached to the 5'-end. After synthesis, the DNA was lyophilized overnight. It was then cleaved from the solid support by incubation at 60^{o} C with concentrated (28-30%) NH_4OH for 12 hours, filtered using CoStar columns, then dried. The dried DNA film was resuspended in phosphate buffer (5 mM phosphate, pH 7, 50 mM NaCl) and HPLC-purified. The DMT (4,4'-dimethoxytrityl) group protecting the 5'- end was then removed by incubation with 80% acetic acid for 45 minutes. The reaction mixture was dried and resuspended in phosphate buffer. The DNA was isolated using HPLC. The purified oligonuceotide was desalted using ethanol precipitation, dried, and the mass was confirmed with Matrix-assisted laser desorption/ionization- time of flight mass spectrometry (MALDI-TOF). Within one week of annealing and use, the dithiolated DNA was reduced by resuspending in 50 mM Tris-HCl, pH 8.4, 50 mM NaCl, 100 mM dithiothreitol (Sigma) for 2 hours. The reduced thiol-modified DNA was then purified by size exclusion chromatography (Nap5 Sephadex, G-25, GE Healthcare) with phosphate buffer as the eluent and subsequently purified using HPLC.

Covalent Coupling of Nile Blue to DNA. DNA was synthesized using ultramild procedures from Glen Research. Phophoramidites compatible with ultramild synthesis were used as well as ultramild Cap A (5% phenoxyacetic anhydride in THF/Pyridine). An NHS-Deoxy-dT phosphoramidite was attached to the 5'- end. After synthesis, the DNA was dried overnight in a lyophilizer. Nile blue perchlorate (Sigma) was then dissolved

(15 mg/ml) in anhydrous N,N-dimethylformamide and activated with 10% v/v DIEA (N,N-Diisopropylethylamine). This Nile blue solution was then incubated with the DNA overnight with gentle shaking. At least 18 hours later, the DNA solid support beads were rinsed with N,N-dimethylformamide then dichloromethane. The DNA was then cleaved from the solid support by incubating with 0.05 M potassium carbonate in methanol for 4 hours at ambient temperature. The DNA was then dried, resuspended in phosphate buffer, and the oligonucleotide was isolated with HPLC. The purified oligonucleotide was desalted using ethanol precipitation, dried, and the mass was confirmed with MALDI-TOF. Nile blue-modified strands were then stored in the dark at -20^{o}C in phosphate buffer until annealing with their complementary strand.

Synthesis of 3'-Dabcyl-DNA. DNA was synthesized using standard phosphoramidites and reagents, with the exception of 3'-Dabcyl modified controlled pore glass (CPG) beads. After synthesis, the DNA was lyophilized overnight. It was then cleaved from the solid support by incubation at ambient temperature with concentrated (28-30%) NH_4OH for 2 hours, spin filtered to remove the solid support beads, then dried. The oligonucleotide was resuspended in phosphate buffer and HPLC-purified. The DMT (4,4'-dimethoxytrityl) group protecting the 5'- end was then removed by incubation with 80% acetic acid for 45 minutes, dried, resuspended in phosphate buffer, and re-purified using HPLC. The purified strand was desalted using ethanol precipitation, dried, and the mass was confirmed with MALDI-TOF.

Annealing Duplex DNA. Duplex DNA for electrochemistry was prepared by first quantifying the complementary strands with UV-Visible spectroscopy, then mixing equimolar (50 μM) complementary strands in 200 μl phosphate buffer. The DNA

solution was then deoxygenated by bubbling argon for at least 5 minutes per ml. Duplex DNA was then annealed on a thermocycler (Beckman Instruments) by initial heating to 90^{o}C followed by slow cooling over 90 minutes.

DNA Sequences:

CG-repeat

3'-GCG CGC GCG CGC GCG C-5'
HS-C_6- 5'-CGC GCG CGC GCG CGC G-3'

C^mG-repeat

3'-GC^mG C^mGC^m GC^mG C^mGC^m GC^mG C^m
HS-C_6- 5'-C^mGC^m GC^mG C^mGC^m GC^mG C^mGC^m G-3'

16bp DNA

3'- TGC AGA GTT GAG TGC A-5'
HS-C_6- 5'-ACG TCT CAA CTC ACG T-3'

30bp DNA (well-matched)

3'-AGA GTT CTT AGC CGT AAT CGA GTT GAC AGT-5'
HS- C_6- 5'- TCT CAA GAA TCG GCA TTA GCT CAA CTG TCA-3'

30bp DNA (C:A mismatch)

3'-AGA GTC CTT AGC CGT AAT CGA GTT GAC AGT-5'
HS- C_6- 5'- TCT CAA GAA TCG GCA TTA GCT CAA CTG TCA-3'

17 bp NB (Well matched)

3'-CGA GTC ATG CTG CAG CT-5'-NB
HS-C_6-5'-GCT CAG TAC GAC GTC GA-3'

17 bp NB (C:A mismatch)

3'-CGA GTC ATG CTG CAG CT-5'-NB
HS-C_6-5'-GCT CAA TAC GAC GTC GA-3'

29 bp NB

3'-CAC CGT CCA GTC AGT ACA TAT GAC GTG AT-5'-NB
HS-C_6-5'-GTG GCA GGT CAG TCA TGT ATA CTG CAC TA-3'

43 bp NB

3'-CGT CAT CCA CTT AGC ACC GTC CAG TCA GTA CAT ATG ACT TGA T-5'-NB
HS-C_6-5'-GCA GTA GGT GAA TCG TGG CAG GTC AGT CAT GTA TAC TGA ACT A-3'

60 bp NB

3'-CAA GTA CTG TAT GCA TGC GTC ATC CAC TTA GCA CCG TCC AGT CAG TAC ATA TGA CTT GAT-5'-NB
HS-C_6-5'-GTT CAT GAC ATA CGT ACG CAG TAG GTG AAT CGT GGC AGG TCA GTC ATG TAT ACT GAA CTA-3'

29 bp Dabcyl

DAB-3'-CAC CGT CCA GTC AGT ACA TAT GAC GTG AT-5'
HS-C_6-5'-GTG GCA GGT CAG TCA TGT ATA CTG CAC TA-3'

The above sequences use the following abbreviations for modifications:

DAB = Dabcyl; NB = Nile Blue; HS-C_6 = hexanethiol linker; C^m = 5-methyl-cytosine

Electrode Fabrication

Single electrode surface fabrication was carried out at UCLA and received as a gift following the general protocol of R. Naaman *et al* (20). P-type oxidized silicon wafers were coated with 25 nm titanium as an adhesive layer, following deposition with 200 nm nickel then 10 nm gold. The surfaces were then cleaved into 1 cm x 1.5 cm rectangles and used following the preparation below (for a total 1 cm^2 exposed surface area for experiments following device assembly). Multiplexed electrode surfaces were fabricated following a modified version of a published protocol (24) using the gold-capped nickel as the electrode material.

DNA-modified electrode preparation

Gold-capped nickel surfaces or multiplexed chips are gently cleaned by rinsing with ultrapure water, acetone, isopropanol, and finally a second rinse with ultrapure water before drying with argon. They are then cleaned with a UVO Cleaner Model 42 (Jelight Co.) for 20 minutes. Immediately after cleaning the surface, a plastic clamp and rubber (BunaN) gasket were affixed to the surface to create a well for liquid and either 50 μM duplex DNA, single stranded DNA, or no DNA in phosphate buffer (pH 7, 5 mM phosphate, 50 mM NaCl, 100 mM $MgCl_2$) to make densely packed films. The DNA was incubated on the surface for 18-24 hours. Once the DNA is on the surface, it cannot be dried without compromising the structure and therefore the measured properties of the film. The solution was then exchanged 5x with 1 μM mercaptohexanol in phosphate buffer (pH 7, 5 mM phosphate, 50 mM NaCl, 5% glycerol) and incubated for 45 minutes.

Lastly the surface was rinsed at least 5x with either phosphate buffer (pH 7, 5 mM phosphate, 50 mM NaCl) for most experiments or tris buffer (pH 7.6, 10 mM Tris, 100 mM KCl, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$) for experiments using dabcyl, and electrochemical experiments were performed immediately afterwards.

Electrochemical measurements

The central well around the electrode surface created by the clamp was filled with one of three buffers prior to electrochemical measurements: a phosphate buffer with $MgCl_2$ (pH 7, 5 mM phosphate, 50 mM NaCl, 10 mM $MgCl_2$), a phosphate buffer without $MgCl_2$ (pH 7, 5 mM phosphate, 50 mM NaCl), and a tris buffer (pH 7.6, 10 mM Tris, 100 mM KCl, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$). The phosphate buffer was used for all experiments, except those containing dabcyl. The phosphate buffer without $MgCl_2$ was only used to collect data with experiments comparing methylated and unmethylated dsDNA. The tris buffer is necessary for both the reduction and oxidation of dabcyl to occur within the potential range that we can scan. Our experiments did not show a statistically significant difference in the magnitude of spin selectivity when comparing DNA sequences of the same length in the different buffers, except in the cases where the increased ionic strength helped prevent electrostatic binding of MB to the phosphate backbone. An AgCl/Ag reference electrode (Cypress) was coated with a solidified mixture of 1% agarose and 3M NaCl in water inside a long, thin pipette tip. The tip was cut so that the salt bridge could connect the electrode to the buffer from the top of the well. A platinum wire used as an auxiliary electrode was also submerged in the buffer from the top of the well. The working electrode contacted a dry part of unmodified gold surface. A grounding wire was

connected to the metallic base of a ring stand. A CH1620D Electrochemical Analyzer (CH Instruments) was used to control the electrochemical experiments.

Magnetic field experiments were conducted using a 6619 Guass surface strength magnetic field neodymium magnet (K&J Magnetics). Alligator clips were replaced with nonmagnetic stainless steel to minimize magnetic interference. All other parts of the assembly were created using plastic to prevent extraneous objects the magnetic field could influence. Additionally, the strong magnet was waved near the potentiostat during operation, with no obvious change in signal that was apparent, to ensure that the magnet was not interfering with the operation of the potentiostat. Each experiment that was conducted for magnetic field pointed up vs down were done by comparing the same surface in the same solution under these varying field directions in order to minimize variability caused by other factors.

Noncovalent Methylene Blue. Solutions of 1μM or 10μM methylene blue were made in phosphate buffer (pH 7, 5 mM phosphate, 50 mM NaCl, 10 mM $MgCl_2$). Cyclic voltammograms were collected using a scan rate of 0.05, 0.1, 0.5, 1, 5, 10, and 20 V/s, which scanned from 0V to -0.55V (vs AgCl/Ag) then back to 0V at least 6 times consecutively for each experiment. The magnetic field direction was then switched, scanned, and switched again multiple times. The first reductive and oxidative sweeps were compared because subsequent scans see a diminishing effect. This attenuation is restored upon waiting for approximately 30 seconds between scans.

Ruthenium Hexammine. A concentration of 10 μM $Ru(NH_3)_6^{3+}$ was added to the phosphate buffer. Cyclic voltammograms were collected at a scan rate of 0.1 V/s, which scanned from 0V to -0.4V (vs AgCl/Ag) then back to 0V at least 6 times consecutively

for each experiment. The magnetic field direction was then switched, scanned, and switched again multiple times. The first reductive and oxidative sweeps were compared.

B-to-Z DNA Experiments. Experiments were conducted with solutions of 1 μM methylene blue in phosphate buffer with and without 10 mM $MgCl_2$. Cyclic voltammograms were collected at a scan rate of 0.1 V/s, which scanned from 0V to -0.4V (vs AgCl/Ag) then back to 0V at least 6 times consecutively for each experiment. The magnetic field direction was then switched, scanned, and switched again at least 4 times. Following these scans, phosphate buffer with 10 mM $MgCl_2$ was placed in the well by exchanging the solution 5x. The surfaces were scanned in a similar manner as before, and then the solution was again exchanged 5x to replace it with a phosphate buffer without magnesium. It was then scanned similarly.

Covalent Nile Blue. Experiments were conducted in phosphate buffer with 4 different lengths of DNA, each with Nile blue covalently tethered at the 5'- end away from the surface. Cyclic voltammograms were collected using a scan rate of 0.05, 0.1, 0.2, 0.5, 1, 2, 3, 4, 5, 7, 10, 11, 12, 13, 14, 15, 17 and 20 V/s, which scanned from 0V to -0.55V then back to 0V 20 times consecutively for each experiment. The magnetic field direction was then switched, scanned, and switched again multiple times. The first reductive and oxidative sweeps were compared.

3'-Dabcyl. Experiments were conducted in tris buffer (pH 7.6, 10 mM Tris, 100 mM KCl, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$) with 1μM methylene blue. Due to the relatively slow rate of redox chemistry with azobenzene, cyclic voltammograms were collected using a scan rate of 10 mV/s so the peak splitting allowed for both the reductive and oxidative peaks to lie within the measurable potential range range. The experiments

scanned from 0V to -0.6V (vs AgCl/Ag) then to 0.5V and repeated scanning between 0.5V and -0.6V 20 times consecutively for each experiment. The magnetic field direction was then switched, scanned, and switched again multiple times. The first reductive and oxidative sweeps were compared.

Calculating the surface concentration of DNA

Surface concentration of DNA was quantified based on the total area of the reductive signal generated from a cyclic voltammogram of electrostatic binding of $Ru(NH_3)_6^{3+}$ to the phosphate backbone of dsDNA. This was done under saturation conditions (10 μM $Ru(NH_3)_6^{3+}$). The following equation was used to relate the reductive signal (Q_{Ru}) to the surface concentration of DNA (Γ_{DNA}):

$$\Gamma_{DNA} = \frac{Q_{Ru}}{n\,F\,A}\,\frac{z}{m}\,N_A$$

In this equation, *A* is the electrode area in cm^2, *F* is the Faraday constant, *n* is the number of electrons per reduction event, *z* is the charge on the $Ru(NH_3)_6^{3+}$, and *m* is the number of base pairs in the duplex DNA. The surface concentration of dilute DNA films was 8±1 pm/cm^2 and dense DNA films was 40±3 pm/cm^2.

Circular Dichroism Spectroscopy

An Aviv 62A DS spectropolarimeter was used to collect circular dichroism (CD) spectra. Data were obtained from samples containing 3 μM $d(^mCG)_8$ or 3 μM $d(CG)_8$ dsDNA in phosphate buffer (5 mM phosphate, 50 mM NaCl, pH7) using a 1.0 mm path length cell (Figure S3). Scans were conducted with samples that were incubated in the presence or absence of 10 mM $MgCl_2$. Data presented in figures represent the average of three scans.

The B-Z transformation is very clearly seen in the CD spectra of the methylated DNA. Upon addition of magnesium ion, there is a large decrease in magnitude of ellipticity at 254 nm and 293 nm with isochromism at 277 nm, which is characteristic of the B-Z transformation. The unmethylated DNA does not show any change in CD spectrum for conditions with and without magnesium ion, which confirms that it remains in the B-form.

Spin Polarization Calculations

The spin polarization (*S*) is defined as:

$$S = \frac{I_+ - I_-}{I_+ + I_-} \quad \textbf{(1)}$$

in which I_+ and I_- are the intensities of the signals corresponding to the spin oriented parallel and antiparallel to the electrons' velocity.(*12)* The spin polarization for electrons travelling *through* dsDNA is calculated assuming that the injected spin polarization is 23% which, using equation 1, results in the total amount of each spin injected assumed to be 0.615 and 0.385, with the majority spin depending on the magnetization direction. (*20)* The amount of charge transferred to the probe (*Q*), which is determined by integrating the current under the reductive or oxidative peak in the cyclic voltammograms, can be related to the injected spin polarization by the following equation:

$$Q = I_+(\eta_+) + I_-(\eta_-) \quad \textbf{(2)}$$

where η_+ and η_- are the yield for the amount of injected spin oriented parallel and antiparallel, respectively, to the velocity of the electrons that reduce the probe compared

to the total amount injected. Therefore the amount of probe reduced can be related as $Q = 0.615\ (Yield_{Up}) + 0.385\ (Yield_{Down})$ for one magnetization and $Q = 0.615\ (Yield_{Down}) + 0.385\ (Yield_{Up})$ for the other.

Modifying equation 1 to solve for the spin polarization through dsDNA gives equation 3.

$$S_{DNA} = \frac{\eta_+ - \eta_-}{\eta_+ + \eta_-} \quad \textbf{(3)}$$

Solving for η_+ and η_- and placing them into equation 3 gives the spin polarization of electrons moving through dsDNA (S_{DNA}).

Our data lead to the calculation that electrons traveling through a densely packed monolayer of 16 bp dsDNA to MB must be at least 22.5±1.2% spin polarized at ambient temperature. Charge transport through 60 bp dsDNA to a covalently tethered NB probe must be at least 55±10% spin polarized. More calculations are presented in Supplemental Table 1.

It should be evident that decreasing the initial spin polarization will increase the calculated spin polarization through dsDNA; therefore these calculated values can be considered lower estimates. If the nickel injected 13% spin polarized electrons, then the electrons passing through 60bp dsDNA would be 100% spin polarized, which could be treated as a theoretical maximum.

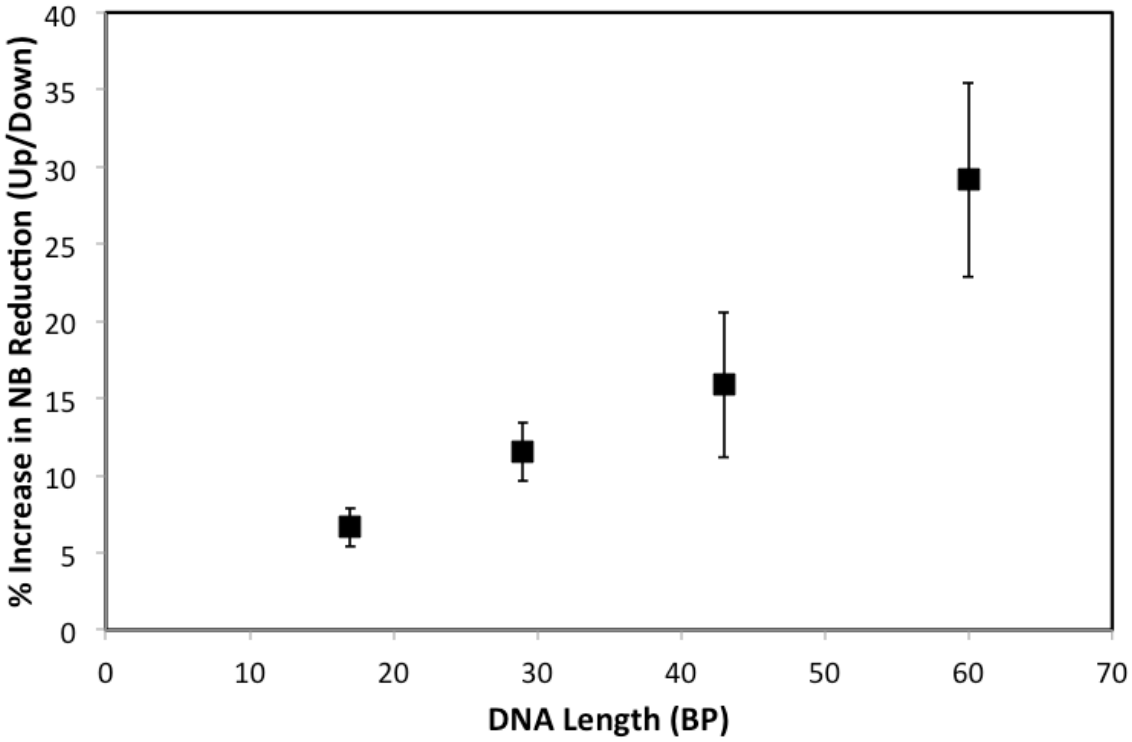

Figure S1. Plot of the magnetic field effect observed for DNA with covalently tethered Nile blue at different oligonucleotide lengths scanned at 20 V/s in phosphate buffer. Each error bar represents the standard error from at least 3 separate surfaces. ANOVA shows a statistically significant difference for the effect being length dependent with $p = 0.017$.

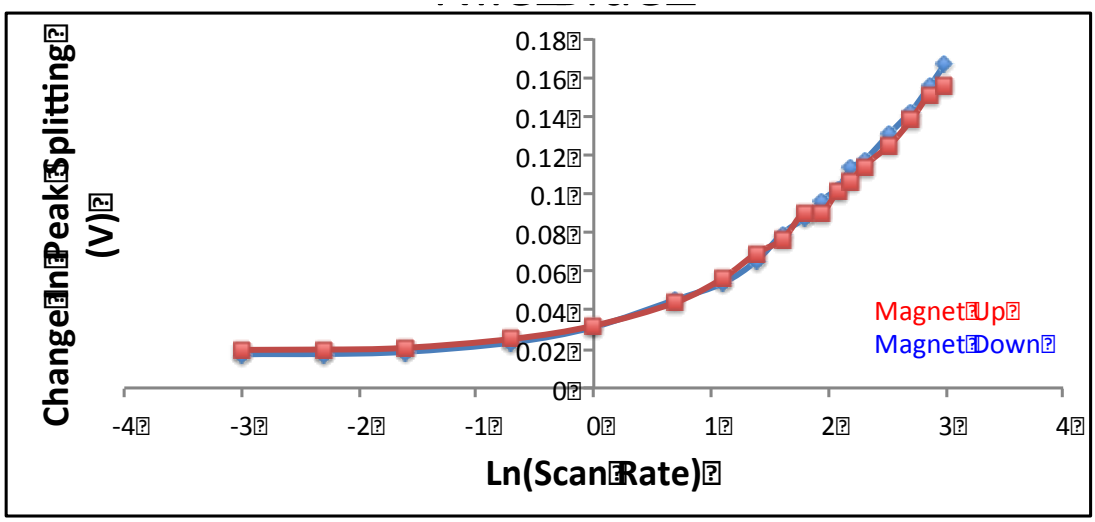

Figure S2. Scan Rate dependence of covalently tethered Nile blue reduction through duplex DNA.

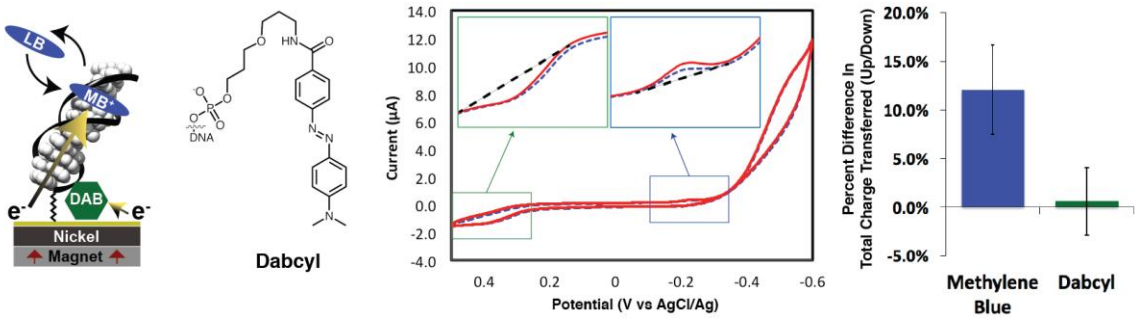

Figure S3. Cyclic voltammetry of 29bp dsDNA with covalently tethered dabcyl and noncovalently intercalated MB. (Left) Cartoon representing the DNA tethered to the surface and the paths the electrons take from the surface to their respective redox probes. (Center Left) Illustration of dabcyl molecule. (Center Right) Representative cyclic voltammogram of DNA with 1 μM MB with the magnetic field pointing towards the surface (red, up) or away from the surface (blue, down). The insets show the dabcyl oxidation (green border) and methylene blue reduction (blue border). The signal centered around -0.5V corresponds to the reduction of dabcyl, while its oxidation appears at 0.3 V. Note that scans were carried out at 10 mV/s given the slow proton-coupled redox reaction. (Right) The difference in cyclic voltammetry data for the reduction of MB (blue), and the oxidation of dabcyl (green) between the two magnetic field directions.

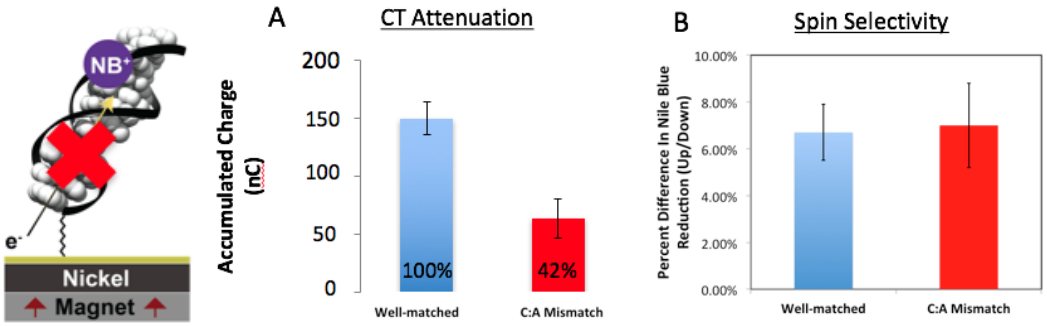

Figure S4. Summary of cyclic voltammetry data for 17bp duplex DNA with a C:A mismatch 6 nucleotides from the surface. **A,** A mismatch (red) has decreased total yield of NB reduction when compared to a well-matched duplex (blue) . **B**, The spin selectivity for probe reduction is the same for DNA with (blue) and without (red) a C:A mismatch.

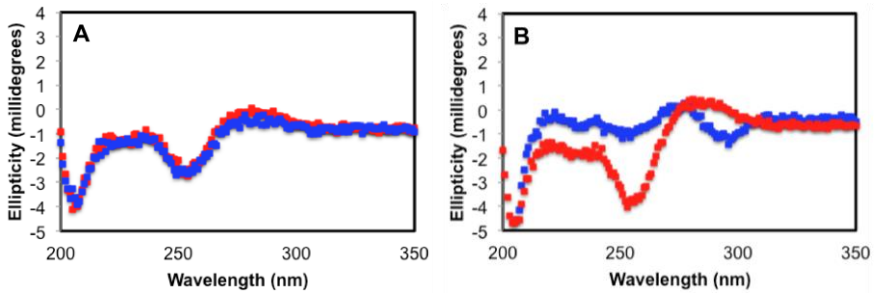

Figure S5. Circular dichroism spectra of [A] unmethylated $d(CG)_8$ and [B] methylated $d(^{m}CG)_8$ in phosphate buffer with [blue] and without [red] $MgCl_2$.

DNA Sequence	Avg Charge Up/Down	SE	Spin Polarization (%)	SE	N
16MB	1.11	0.01	23	2	3
30MB	1.15	0.01	30	2	3
17NB	1.07	0.01	15	2	3
17NB (Mismatch)	1.07	0.02	15	4	3
29NB	1.12	0.02	25	4	3
43NB	1.16	0.04	32	7	3
60NB	1.29	0.06	55	10	4
30mCG (Before)	1.18	0.03	36	5	6
30mCG (Mg)	0.91	0.02	-19	4	6
30mCG (After	1.18	0.02	36	4	6
16mCG (Before)	1.1	0.02	21	4	12
16mCG (Mg)	0.95	0.01	-11	2	12
16mCG (After)	1.07	0.01	15	2	12
30CG (Before)	1.16	0.02	32	4	8
30CG (Mg)	1.1	0.04	21	8	8
30CG (After)	1.13	0.03	27	6	8
16CG (Before)	1.1	0.03	21	6	4
16CG (Mg)	1.1	0.03	21	6	4
16CG (After)	1.09	0.01	19	2	4
29DAB (MB)	1.18	0.07	36	11	7
29DAB (Dabcyl)	1.05	0.08	10	16	7

Table S1. Summary of all spin polarization data. Data shown is the average amount of charge up/down and its corresponding standard error and number of independent sets of scans, N.