Supplementary Information

Electrical Detection of TATA Binding Protein at DNA-Modified Microelectrodes

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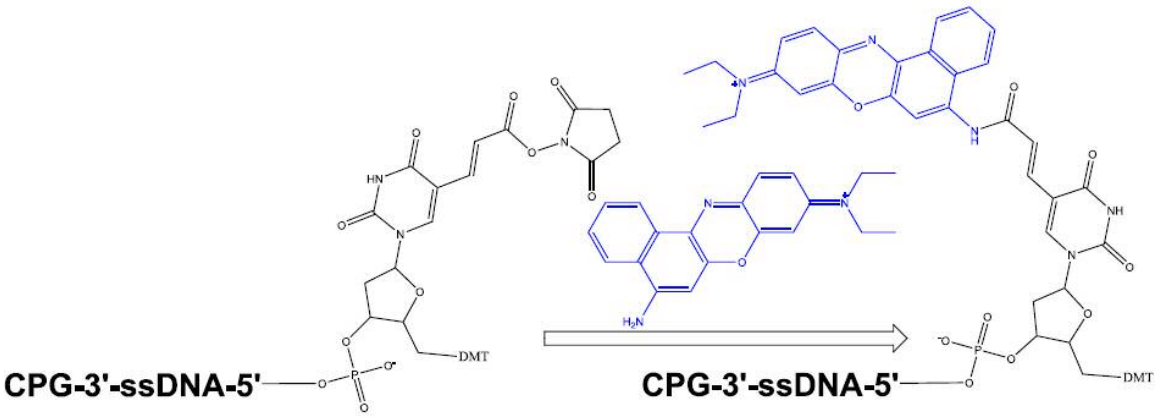
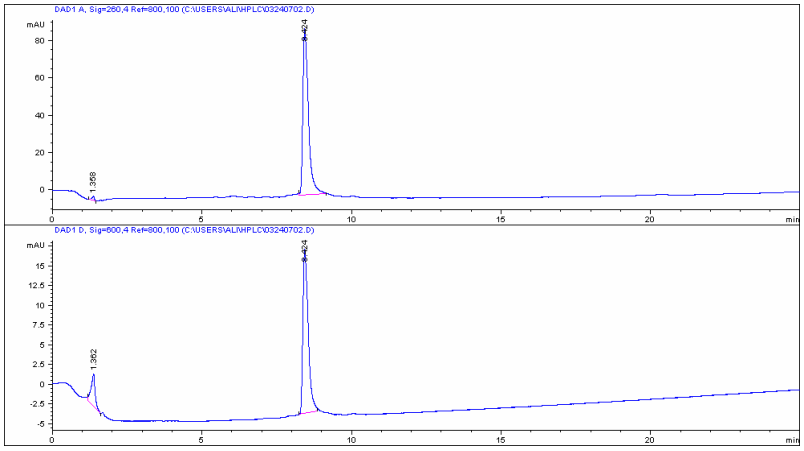
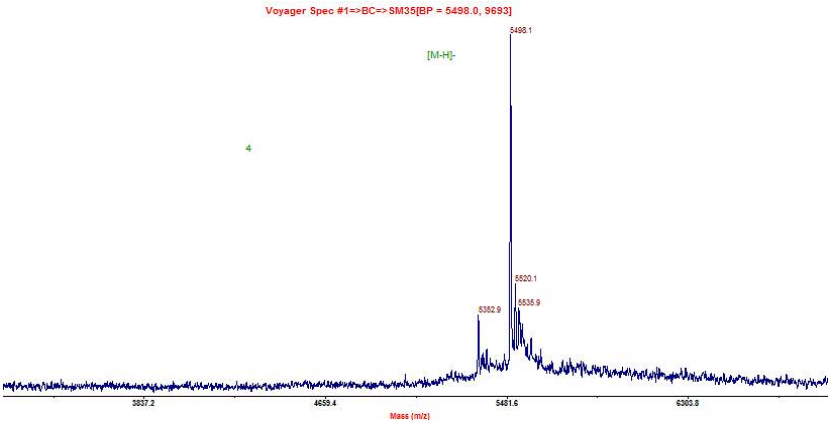


Supporting Figure 1: Schematic illustration of the coupling of Nile Blue to the NHS-Carboxy-dT modified DNA on solid support. The solvent was 9:1 N,N-dimethylformamide/N,N-diisopropylethylamine. The overall yields of the reaction ranged from 30% to 80%.

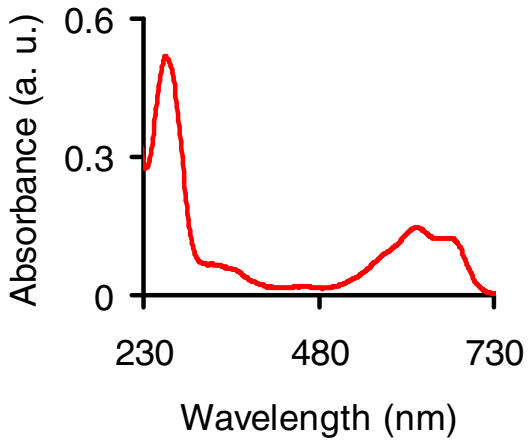
A.



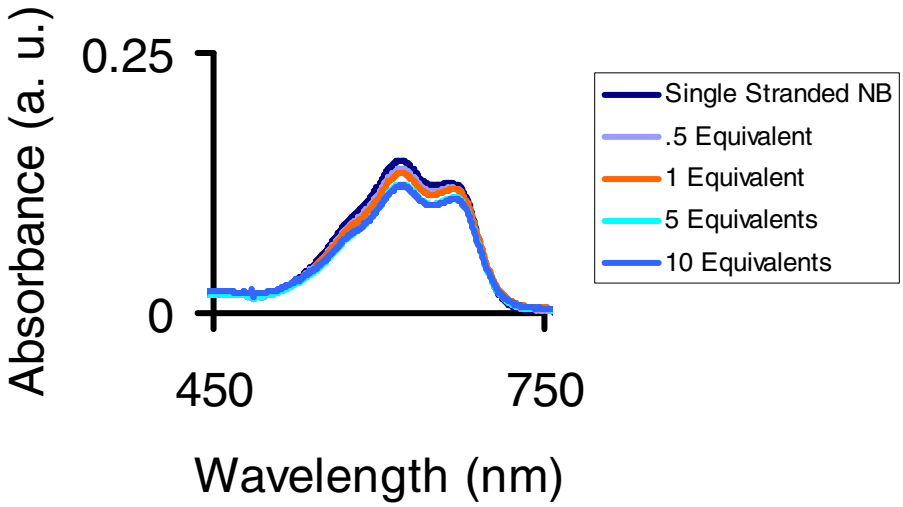
B.



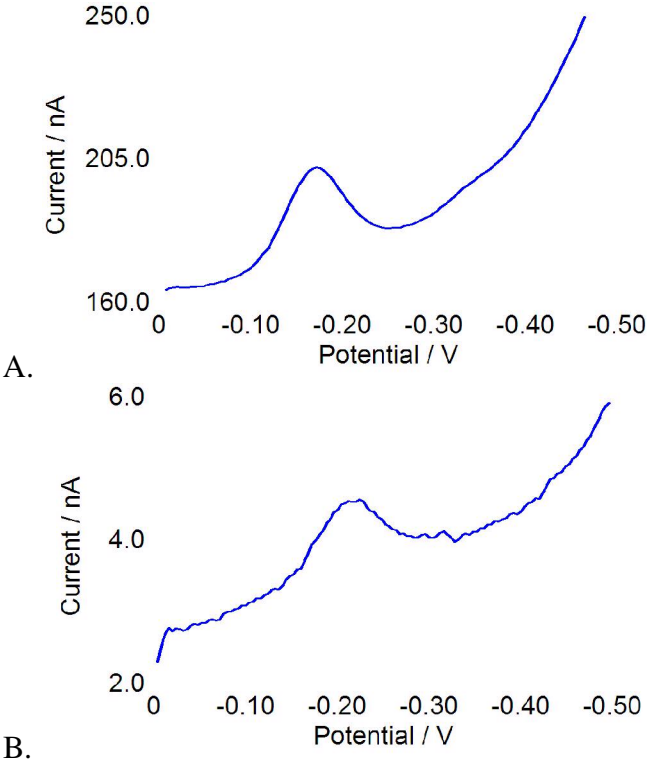
C.



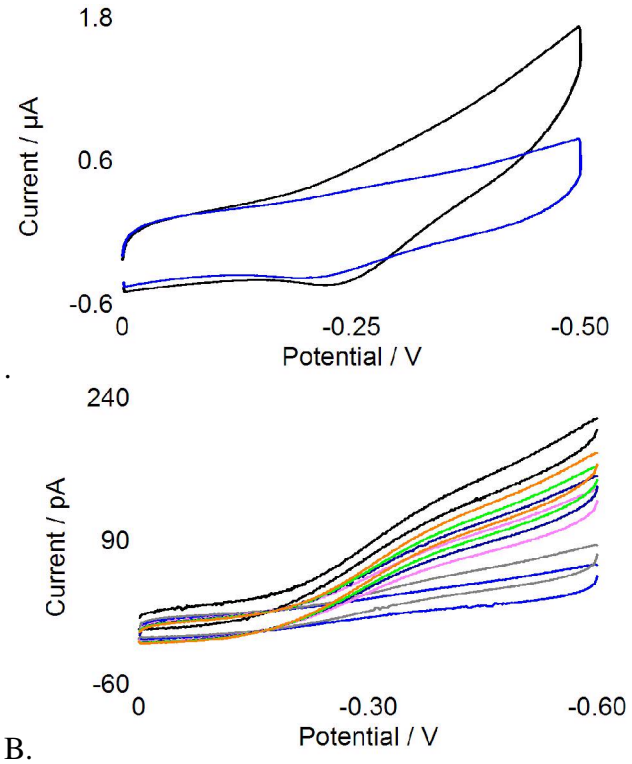
Supporting Figure 2: Characterization and purification of the NB-DNA conjugate. (A) Analytical HPLC chromatogram of the pure NB-DNA conjugate with an acetonitrile gradient of 5% to 75% over 30 minutes. (B) MALDI mass spectrometry of the pure NB-DNA conjugate with the MW_{found}= 5498 amu and $MW_{expected}$=5499 amu. (C) Corresponding UV-visible absorption spectrum showing the Nile Blue peak at 600 nm and the DNA peak at 260 nm.



Supporting Figure 3: UV-visible absorption spectrum of the Nile Blue modified ssDNA (sequence 5'-UGC GTG CTT TAT ATC TC -3') recorded before and after addition of increasing equimolar amounts of the complement. Significant hypochromicity is observed for the Nile Blue moiety, the location of which is indicated by the U.

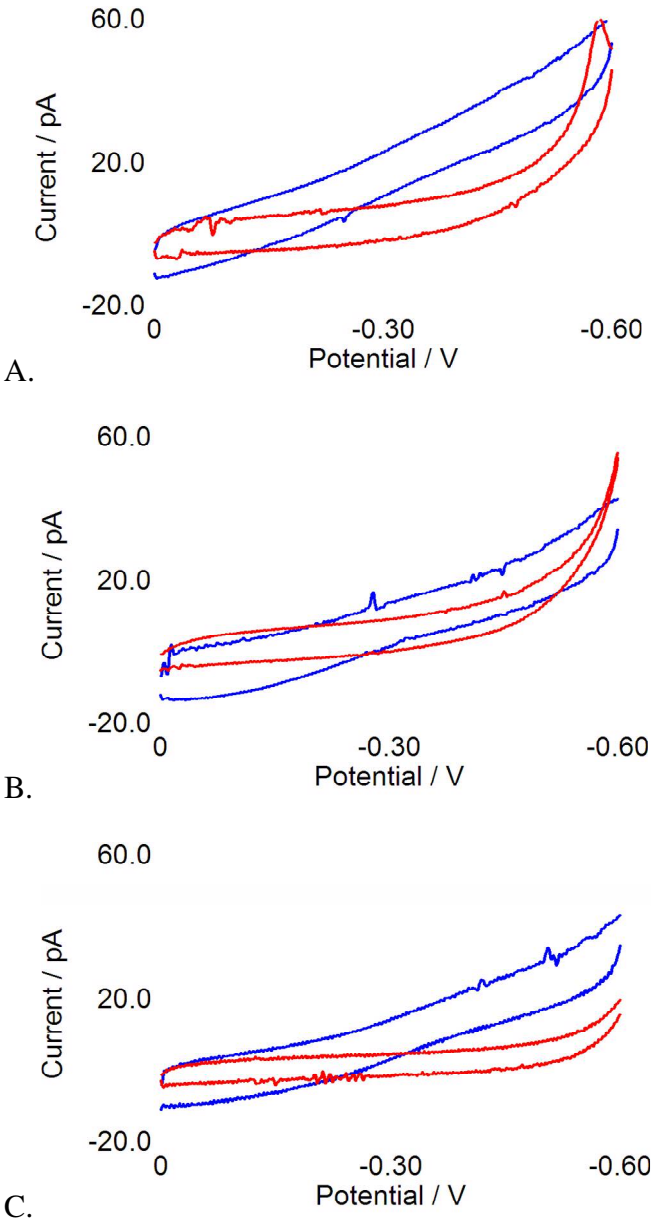


Supporting Figure 4: Square wave voltammetry of a NB-DNA modified Au on silicon electrodes of 130 µm radius (A) and 13 µm radius (B) in pH = 7 phosphate buffer containing 5 mM P_i and 50 mM NaCl. Note that the two electrodes were fabricated together in parallel and run minutes apart yet still did not yield identical potentials.

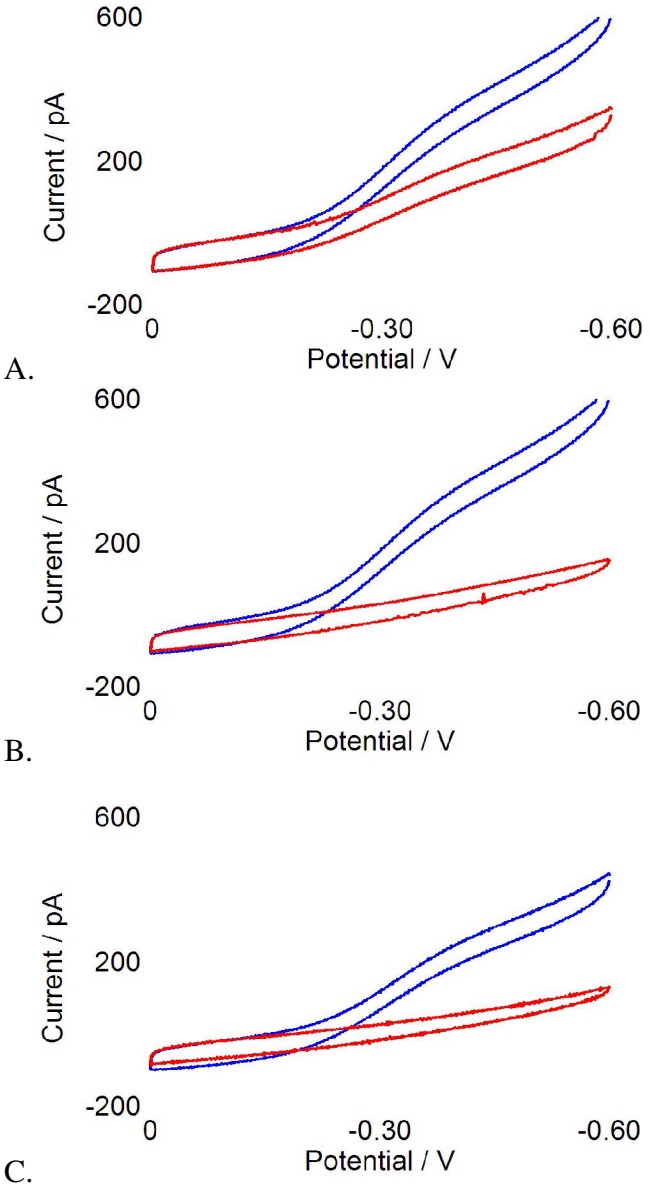


B.

Supporting Figure 5: The effect of oxygen on cyclic voltammetry at (A) a macroelectrode and (B) a 10 µm diameter microelectrode at 50 mV/s. Voltammetry in oxygen saturated buffers is shown in black with voltammetry after ten to fifteen more minutes of vigorous argon bubbling into the solution in blue. Trace amounts of oxygen have a more significant effect on voltammetry observed at microelectrodes. The gray to orange traces in B represent successive voltammograms taken on the same electrode open to air in an initially degassed solution with a slow, steady increase observed over thirty minutes.



Supporting Figure 6: Cyclic voltammetry of a NB-DNA modified 10 µm diameter electrode at 50 mV/s before addition of TBP in blue and after addition of 300 nM TBP in red. Initial background voltammetry of the electrode is shown in (A) with significant signal loss observed after addition of TBP. The electrode is subsequently rinsed in succession (5 minutes each) with 1 M KCl in MQ H_2O, 1 M KCl in Tris buffer, and TATA storage buffer. Background voltammograms are recorded for the same electrode before it is used to detect TBP again (B). The electrode can then be taken through another KCl rinse cycle before being used for detection a third time (C).



Supporting Figure 7: Cyclic voltammetry of NB-DNA modified 25 µm diameter electrodes at 50 mV/s before addition of TBP in blue and after addition of TBP in red. The signal attenuation shown is for addition of (A) 3 nM, (B) 15 nM, and (C) 30 nM.

Protein Preparation

(1) Human TATA binding protein was custom ordered from ProteinOne, Inc. in pH = 7, 5 mM NaP_i, 50 mM NaCl, 4 mM $MgCl_2$, 4 mM spermidine, 50 µM EDTA, 10 % glycerol buffer. The protein concentration was confirmed using the Bradford Assay Kit from Bio-Rad Laboratories. Stock solutions of the protein were stored at -80ºC and aliquoted under an argon atmosphere. The TATA binding protein utilized for detection experiments was taken through no more than two freeze/thaw cycles.

(2) Bovine serum albumin was purchased from New England Biolabs, Inc. and utilized for experiments as received.

(3) Endonuclease III was prepared according to established literature procedures (see Boal, A. K.; Yavin, E.; Lukianova, O. A.; O'Shea, V. L.; David, S. S.; Barton, J. K. *Biochemistry*, **2005**, *4*, 8397.). The enzyme was quantified by UV-visible spectroscopy as described in the references therein.

(4) Bam HI Methyltransferase was purchased from New England Biolabs, Inc. Prior to experiments, the protein was dialyzed overnight in pH = 7, 5 mM NaP_i, 50 mM NaCl, 4 mM $MgCl_2$, 4 mM spermidine, 50 µM EDTA, 10 % glycerol buffer to remove excess dithiothreitol.