Supplemental Information for "A Multiplexed, Two-Electrode Platform for Biosensing based on DNA-Mediated Charge Transport"

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Methods and Materials

Preparation of Surfaces and First Alkanethiol Monolayers.

Gold surfaces were polished with 0.05 μm alumina slurries (Buhler) before monolayer assembly. Mixed monolayers were then formed on the substrate plate by self-assembly of 100 mM 12-azidododecane-1-thiol (C_{12} thiol azide) and 100 mM 11-mercaptoundecyl-phosphoric acid from an ethanolic solution. Surfaces were incubated in the thiol solution for 18-24 h, followed by rinsing with ethanol and phosphate buffer (5 mM phosphate, 50 mM NaCl, pH 7.0).

DNA Synthesis and Purification.

Hexynyl-labeled oligonucleotides were synthesized on an Applied Biosystems 3400 DNA synthesizer, modified at the 5` end with a C6-alkyne reagent purchased from Glen Research, Inc. Complementary unmodified strands were purchased from IDT. DNA strands modified with Nile Blue at the 5` terminus were prepared as previously reported.[S1] Briefly, DNA was synthesized with ultramild reagents (Glen Research, Inc) to prevent Nile Blue degradation, and 5-[3-acrylate NHS ester]-deoxy uridine was incorporated as the 5` terminal base. With DNA on the solid support, 10 mg/mL Nile Blue perchlorate in 9:1 *N,N*-dimethylformamide/*N,N*-diisopropylethylamine (Sigma Aldrich) was added and allowed to shake for 24 h. Beads were washed three times each with *N,N*-dimethylformamide, methanol and acetonitrile. The DNA was removed from the solid support with 0.05 M potassium carbonate in methanol at ambient temperature

for 24 h. Preparation of all oligonucleotides followed a reported protocol. For non-ultramild syntheses, DNA was deprotected and cleaved from the solid support with ammonium hydroxide (60° C for 12 h). Following a preliminary round of high-performance liquid chromatography (HPLC) on a PLRP-S column (Agilent), oligonucleotides were treated with 80% acetic acid in water for 20 minutes. Each oligonucleotide was again purified by HPLC using a gradient of acetonitrile and 50 mM ammonium acetate. Oligonucleotides were then desalted by ethanol precipitation and quantified by ultraviolet-visible spectrophotometry based on their extinction coefficients at 260 nm (IDT Oligo Analyzer). Oligonucleotide masses were verified by matrix-assisted laser desorption (MALDI) mass spectrometry. DNA duplexes were formed by thermally annealing equimolar amounts of single-stranded oligonucleotides in deoxygenated phosphate buffer (5mM phosphate, 50 mM NaCl, pH 7.0) at 90° C for 5 minutes followed by slowly cooling to 25° C.

The following sequences were prepared:

Well Matched
Alkyne: H-C_2-$(CH_3)_6$-5'-GCT CAG TAC GAC GTC GA-3'
Complement: 3'-CGA GTC ATG CTG CAG CT-5'

Mismatched
Alkyne: H-C_2-$(CH_3)_6$-5'-GCT CAG TA*C* GAC GTC GA-3'
Complement: 3'-CGA GTC AT*A* CTG CAG CT-5'

TBP Binding Sequence:
Alkyne: H-C_2-$(CH_3)_6$-5'-GGC GTC **TAT A**AA GCG ATC GCG A-3'
Complement: 3'-CCG CAG **ATA T**TT CGC TAC CGC T-5'

COPG Binding Sequence:
Alkyne: H-C_2-$(CH_3)_6$-5'-AAC CG**T GCA** CTC AA**T GCA** ATC-3'
Complement: 3'-TTG GC**A CGT** GAG TT**A CGT** TAG-5'

The location of the mismatch is indicated in italics and with an underline, and the protein binding sites are shown in bold.

TBP and CopG Experiments.

TATA-Binding Protein (TBP) was purchased from ProteinOne, and CopG was purchased from Origene. Both proteins were stored at -80 °C until use. MicroBiospin 6 columns (BioRad) were used to exchange the shipping buffer for Tris buffer (10 mM Tris, 100 mM KCl, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.6). Prior to electrochemical measurements with CopG and TBP, electrodes were incubated with 1 μM Bovine serum albumin (BSA) for 30 min, followed by rinsing with Tris buffer (10 mM Tris, 100 mM KCl, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.6). Protein solutions (4 μL) were added to each electrode and incubated for 20 minutes at ambient temperature prior to measurement.

References

S1. Gorodetsky, A. A.; Ebrahim, A.; Barton, J. K. Electrical Detection of TATA Binding Protein at DNA-Modified Microelectrodes. *J. Am. Chem. Soc.* **2008**, *130*, 2924-2925.

Figure Captions

Figure S1: Electrochemistry of $[Cu(phendione)_2]^{2+}$. A cyclic voltammogram (CV) of $[Cu(phendione)_2]^{2+}$ was obtained in degassed Tris buffer (10 mM Tris, 100 mM KCl, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.6) with a glassy carbon working electrode using a scan rate of 0.1 V/s against an AgCl/Ag reference electrode.

Figure S2: Nyquist plots of electrochemical impedance spectroscopy of differentially formed monolayers. Shown are results for a bare gold electrode (black), a mixed monolayer of azide and phosphate-terminated thiols (green), a DNA monolayer formed from $[Cu(phendione)_2]^{2+}$ catalyst activation from the secondary electrode (blue), and a DNA monolayer formed from the catalyst activation at the primary, substrate electrode (red). Conditions used for impedance spectroscopy were 400 μM ferrocyanide in phosphate buffer (5 mM phosphate, 50 mM NaCl, pH 7.0).

Figure S3: Optimizing the spacer height. Eight Teflon spacers of different heights were tested for electrochemical signal and mismatch discrimination. The spacer between the two electrode arrays establishes the gap between the two electrodes (left). The current from constant current amperometry obtained as a function of spacer height (center) is maximized with the 127 μm spacer (red asterisk). Mismatch discrimination as a function of spacer height (right) is reported as a ratio of the mismatched signal to the well matched signal, with maximal discrimination also observed with the 127 μm spacer (red asterisk). All electrochemistry was conducted in Tris buffer (10 mM Tris, 100 mM KCl, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.6) with 4 μM methylene blue and 300 μM $K_3[Fe(CN)_6]$. 18-mer well matched and mismatched DNA was used.

Figure S1

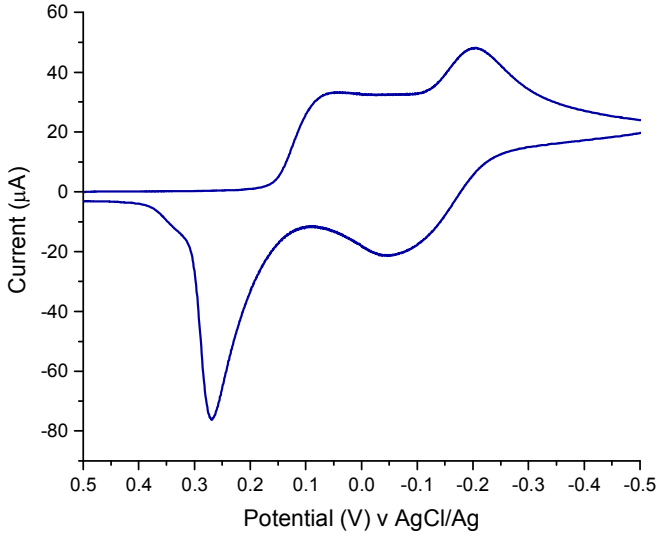

60
40
20
0
-20
-40
-60
-80
Current (μA)
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
Potential (V) v AgCl/Ag

Figure S2

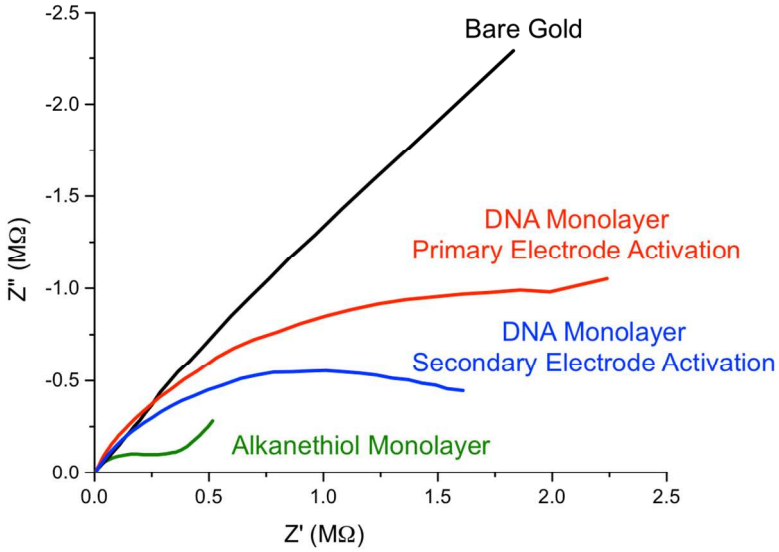
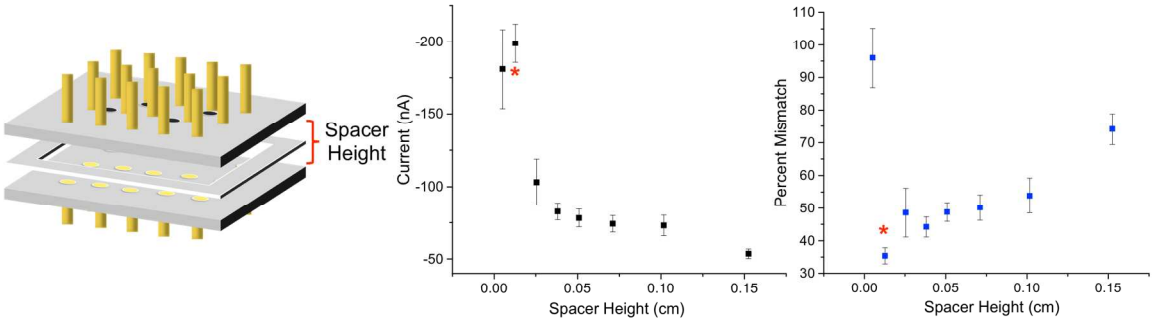

Bare Gold
DNA Monolayer
Primary Electrode Activation
DNA Monolayer
Secondary Electrode Activation
Alkanethiol Monolayer
Z'' (MΩ)
Z' (MΩ)
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
0.0
0.5
1.0
1.5
2.0
2.5

Figure S3


Spacer
Height
Current (nA)
-200
-150
-100
-50
0.00
0.05
0.10
0.15
Spacer Height (cm)
Percent Mismatch
110
100
90
80
70
60
50
40
30
0.00
0.05
0.10
0.15
Spacer Height (cm)