Supporting Information

Fabrication of an Electrochemical DNA Sensor Array via Potential-assisted "Click" Chemistry

Socrates Jose P. Cañete and Rebecca Y. Lai*
Department of Chemistry, University of Nebraska-Lincoln
Lincoln, NE 68588-0304 USA
**Correspondence should be addressed to Rebecca Y. Lai*
E-mail: rlai2@unl.edu
Phone: (402) 472-5340
Fax: (402) 472-9402

MATERIALS AND METHODS

Materials and Instrumentation. The reagents 11-mercapto-1-undecanol (C11-OH), sulfuric acid (H_2SO_4), trizma base, copper sulfate ($CuSO_4$), ethanol (EtOH), TWEEN® 20 (Tween), dimethyl sulfoxide (DMSO) and tris[(1-benzyl-1 H-1,2,3-triazol-4-yl)methyl]amine (TBTA) were used as received (Sigma-Adrich, St. Louis, MO). 11-azido-1-undecanol (C11-N_3) was obtained from ProChimia Surfaces (Sopot, Poland) and was used without further purification. The surrogate probe, alkyne-modified C_7-MB, was obtained from Biosearch Technologies (Novato, CA) (SI-1). All other chemicals were of analytical grade. All the solutions were made with deionized water (DI water) purified through a Milli-Q system (18.2 MΩ·cm, Millipore, Bedford, MA). Physiological buffer solution (Phys2, pH 7.4) consisted of 20 mM Tris, 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$ adjusted to pH 7.4 with hydrochloric acid.

For the E-DNA sensor array, dual-modified oligonucleotides obtained from Biosearch Technologies, Inc. (Novato, CA) were used as the DNA probes (SI-5). A methylene blue (MB) redox reporter was conjugated to the 3' end of the oligonucleotide via succinimide ester coupling to a 3'-amino modification. The probe is also modified with a C3-alkyne at the 5'-end for conjugation to the surface azide and the probe sequences are as follows:

K-ras probe: 5'-alkyne-CCGTTACGCCACCAGCTCCAAACGG-Methylene Blue-3'
p53 probe: 5'-alkyne-CCGTTCCTCCGGTTCATGCCAACGG-Methylene Blue-3'
Brca2 probe: 5'-alkyne-CCGTTACGGCCCTGAAGTACAACGG-Methylene Blue-3'

The target DNA sequences were purchased from Integrated DNA Technologies (Coralville, IA) and the sequences are as follows:

K-ras target: 5'-TTGGAGCTGGTGGCGTA-3'
p53 target: 5'-TGGCATGAACCGGAGGA-3'
Brca2 target: 5'-TGTACTTCAGGGCCGTA-3'

Electrochemical measurements were performed at room temperature (22±1°C) using a CHI 1040A Electrochemical Workstation (CH Instruments, Austin, TX). Cyclic voltammetry (CV) and alternating current voltammetry (ACV) were both employed in this study. Polycrystalline gold disk and glassy carbon electrodes (CH Instruments, Austin, TX) were used as working electrodes. The counter electrode used was a platinum wire electrode and a Ag/AgCl (3 M KCl) electrode served as the reference electrode, both from CH Instruments (Austin, TX). Prior to sensor fabrication, the gold electrodes were polished with a 0.1 μm diamond slurry (Buehler, Lake Bluff, IL), rinsed with DI water and sonicated in a low power sonicator for approximately five minutes to remove bound particulates. They were then electrochemically cleaned by a series of oxidation and reduction cycles in 0.5 M H_2SO_4 and in 0.05 M H_2SO_4. The real area of each electrode was determined from the charge associated with the gold oxide stripping peak obtained after the cleaning process.

Cyclic Voltammetric Studies of Cu^{2+} in Presence of TBTA in 1:1 DMSO/H_2O

Glassy carbon electrodes were polished using 0.1 μm diamond slurry (Buehler, Lake Bluff, IL), rinsed with DI water, sonicated for approximately five minutes, and were dried under nitrogen (N_2). Gold disk electrodes were cleaned accordingly to a similar procedure, with the addition of an electrochemical cleaning step. An azide-containing self-assembled monolayer (SAM) was developed on the electrode by first incubating the electrode in a solution consisted of 67 mM C11-OH and 133 mM C11-N_3 for 30 minutes. The electrodes were subsequently immersed in 5 mM C11-OH for another 4 hrs to complete the SAM formation. Cyclic voltammograms were recorded at a scan rate of 100 mV/s in a deacerated DMSO/H_2O solution containing 75 mM $CuSO_4$, 150 mM TBTA and 0.1 M NaCl.

"Click" Potential Optimization

Three different applied potentials were chosen (i.e., -300, -400 and -500 mV) to optimize the number of captured probes. The azide-containing SAM-modified electrodes were first immersed in a DMSO/H_2O solution containing 75 mM $CuSO_4$, 150 mM TBTA, 0.1 M NaCl and 3.6 μM alkyne-modified C_7-MB. The assigned potential will then be applied to the electrodes for a fixed 30 minutes. To remove physically adsorbed probe DNA, the electrode was rinsed thoroughly with DI water, 5% Tween, EtOH and then again in the reverse order. Electrochemical characterization of the films was performed using ACV. AC voltammograms were recorded in a Phys2 buffer from -0.05 V to -0.5 V vs. Ag/AgCl with a 10 Hz, 25 mV AC potential.

"Click" Efficiency Differences Between C_7-MB and the *K-ras* DNA Probe

The C_7-MB and *K-ras* probes were conjugated on azide-modified electrodes via potential-assisted "click" chemistry at the optimized "click" potential of -500 mV. The "click" mixture used was a 1:1 DMSO/H_2O solution containing 75 mM $CuSO_4$, 150 mM TBTA, 0.1 M NaCl and 3.6 μM alkyne-modified C_7-MB. Electrochemical characterization of the resultant films was performed using ACV. AC voltammograms were recorded in a Phys2 buffer from -0.05 V to -0.5 V vs. Ag/AgCl with a 10 Hz, 25 mV AC potential.

Surface Probe Density Calculations

The number of electroactive probes (e.g., C_7-MB, *K-ras* DNA probes) on the electrode surface, N_{tot} was determined using a previously established relationship with ACV peak current described in eq. 1:[1, 2]

$$I_{avg}(E_0)=2nfFN_{tot}\sinh(nFE_{ac}/RT)/[\cosh(nFE_{ac}/RT)+1] \quad (1)$$

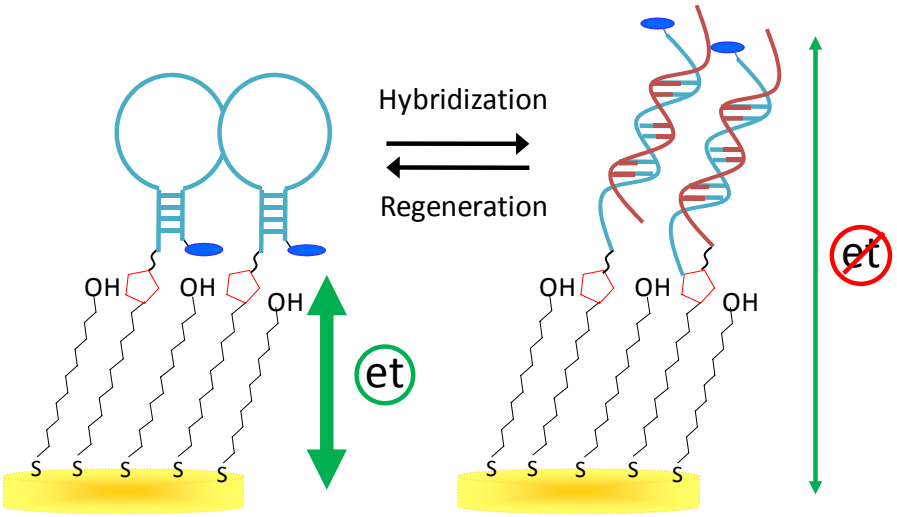
Where $I_{avg}(E_0)$ is the average AC peak current in a voltammogram, n is the number of electrons transferred per redox event (n = 2, MB label), F is the Faraday current, R is the universal gas constant, T is the temperature, E_{ac} is the peak amplitude, and f is the frequency of the applied ACV. The surface density of probes was measured in the number of electroactive probes per unit area.

Fabrication of a 3-pixel E-DNA Sensor Array

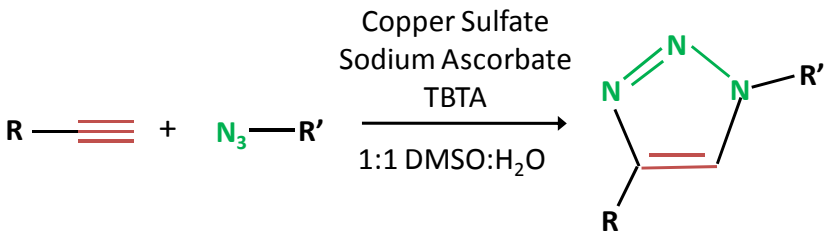
The 3-pixel gold electrode array used in this study was fabricated using standard microfabrication techniques and the procedures were published elsewhere.[3] The electrode array was modified with an azide-containing SAM as described above. The electrode array was then immersed in a "click" mixture containing 1.4 mM TBTA, 0.44 mM $CuSO_4$, 4.3 μM *Brca2* probe, 0.1 M NaCl, and 2.7 mM $MgCl_2$. Only Pixel 1 of the array was biased at -500 mV (vs. Ag/AgCl reference electrode) for 30 minutes, the remaining two pixels were not connected to the potentiostat (i.e. open circuit potential). The array was then removed from the solution, rinsed with DI water, 5% Tween, EtOH and then again in the reverse order. Next, the array was immersed in the second "click" mixture containing the *p53* probe for 30 minutes, with Pixel 2 biased at -500 mV while keeping Pixel 1 and 3 disconnected from the potentiostat. After rinsing, the electrode array was incubated in the third "click" mixture containing the *K-ras* probe. Pixel 3 was held at -500 mV for 30 minutes, whereas Pixels 1 and 2 were disconnected.

Target Interrogation and Sensor Regeneration

The resultant 3-pixel E-DNA sensor array was allowed to equilibrate in a Phys2 buffer until a stable baseline was observed in the AC voltammogram. Sensor interrogation with 1μM target DNA was then performed in a sequential manner according to the following order: *Brca2*, *p53*, *K-ras*. To determine the hybridization kinetics, each sensor in the array was interrogated at different time intervals in the target solution until a stable peak current was obtained (i.e. signal saturation). The ratio between the stabilized peak current in the target DNA solution and the peak current in the target DNA-free solution was used to calculate the signal suppression caused by the target. Post hybridization, sensor regeneration was achieved by rinsing for 30 seconds with deionized water. The sensor array was placed back into a Phys2 buffer to determine the degree of sensor regeneration.



SI-1. The E-DNA sensor consists of a redox-labeled stem-loop DNA probe covalently attached to an interrogating electrode. The E-DNA signal arises due to the binding-induced change in the conformation of the stem-loop probe and the efficiency with which the attached redox label transfers electrons to the electrode. In the absence of target, the stem-loop structure holds the redox label in proximity to the electrode, enabling efficient electron transfer. Upon hybridization with the complementary target DNA, the double-stranded conformation of the probe DNA forces the redox label away from the electrode, impeding electron transfer and leading to a detectable reduction in redox current.



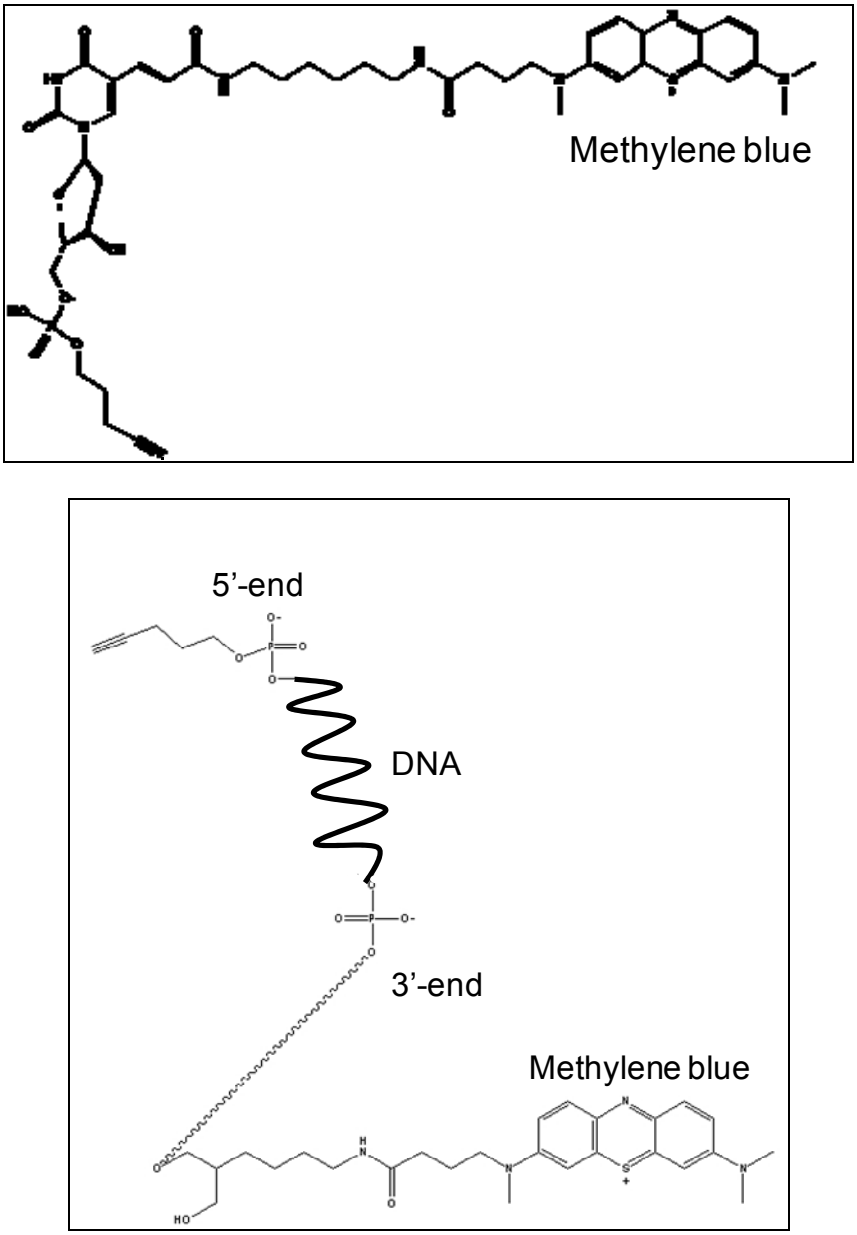
SI-2. Sharpless "click" chemistry.

For the "click" chemistry-based E-DNA Sensor:

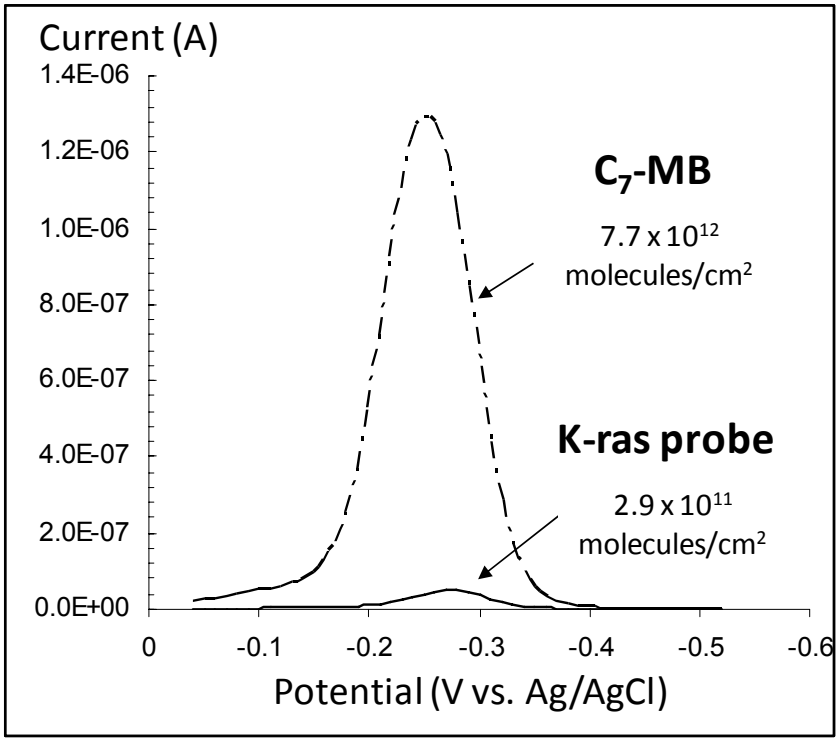
R = C3-DNA-C7-methylene blue

R' = HS-$(CH_2)_{11}$

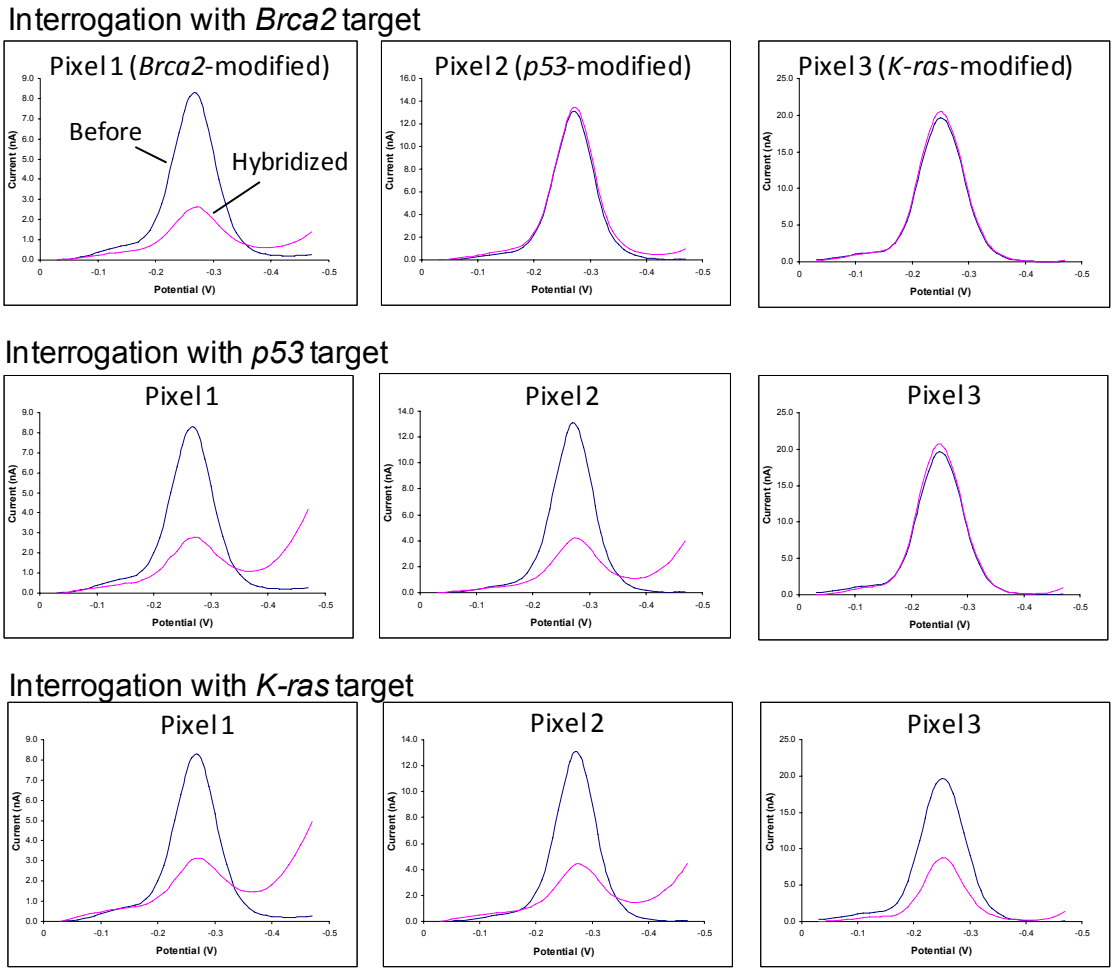
TBTA = tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine



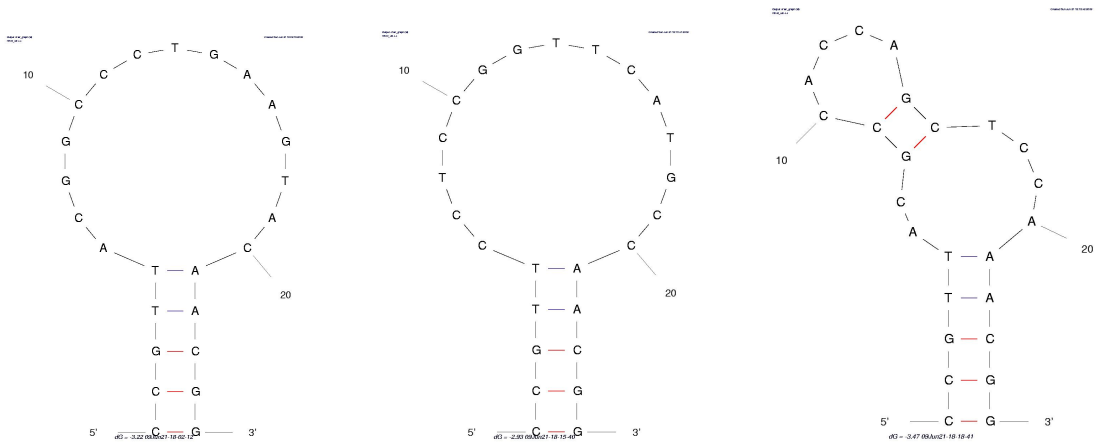
SI-3. Structures of the C_7-MB probe (top) and the alkyne-modified DNA probe (bottom).



SI-4. AC voltammograms of C_7-MB (dotted line) and *K-ras* probe-modified E-DNA sensor (solid line) fabricated via potential-assisted "click" chemistry under the same experimental condition. The voltammograms were recorded in a Phys2 buffer from -0.05 V to -0.52 V vs. Ag/AgCl with a 10 Hz, 25 mV AC potential.



SI-5. AC voltammograms obtained from the 3-pixel E-DNA sensor array. Shown are the sensors' responses to the added target DNA. The sensors were interrogated in sequence, in which the *Brca2* target was first added (top). A large signal suppression was observed in Pixel 1, the pixel that was selectively modified with the *Brca2* DNA probes. No signal suppression was observed with the two pixels that were modified with *p53* and *K-ras* probes. The *p53* target DNA was added once hybridization was complete for the *Brca2*-modified pixel (center). The *K-ras* target was the last to be added (bottom).



SI-6. The lowest energy structures of the *Brca2* (left), *p53* (center) and *K-ras* (right) DNA probes as predicted by m-Fold.[4] The simulation was performed at 23°C and in presence of 0.15 M Na^+ and 0.002 M Mg^{2+}. As observed, the *K-ras* probe has a mini-loop structure that is absent in the *Brca2* and *p53* probes. This structure affects target hybridization both kinetically and thermodynamically.

References

1 O'Connor, S.D.; Olsen, G.T.; Creager, S.E., *J. Electroanal. Chem.*, **1999**, *466*, 197.
2 Sumner, J.J.; Weber, K.S.; Hockett, L.A.; Creager, S.E., *J. Phys. Chem. B*, **2000**, *104*, 7449.
3 Lai, R. Y.; Lee, S-H.; Soh, H. T.; Plaxco, K. W.; Heeger, A. J., *Langmuir* **2006**, *22*, 1932.
4 http://mfold.bioinfo.rpi.edu/cgi-bin/dna-form1.cgi