Supporting Information

Folding-based electrochemical DNA sensor fabricated by "click" chemistry†

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MATERIALS AND METHODS

Materials and Instrumentation. The reagents 11-mercapto-1-undecanol (C11-OH), 8 M guanidine hydrochloride (GHCl), tris-(2-carboxyethyl) phosphine hydrochloride (TCEP), trizma base, copper sulfate ($CuSO_4$), sodium ascorbate, ethanol (EtOH), dimethyl sulfoxide (DMSO) and tris[(1-benzyl-1 H-1,2,3-triazol-4-yl)methyl]amine (TBTA) were used as received (Sigma-Aldrich, St. Louis, MO). Azide-terminated undecyl disulfide was obtained from Asemblon, Inc. (Redmond, WA) and was used without further purification. Human whole blood purchased from Innovative Research (Novi, MI) was used as received. 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 both "click" chemistry-based and conventional E-DNA sensors, a methylene blue (MB)-modified stem-loop oligonucleotide complementary to the *K-ras* gene was used as the probe with the sequence 5'-CCGTTACGCCACCAGCTCCAAACGG-$(CH_2)_7$-NH-MB-3'. (Biosearch Technologies, Inc. Novato, CA). The MB redox moiety was conjugated to the 3' end of the oligonucleotide via succinimide ester coupling to a 3'-amino modification. The "click" chemistry probe DNA is modified with a C3-alkyne at the 5'-end for conjugation to the surface azide and the probe sequence is as follows:

5' HC≡C-$(CH_2)_3$-CCGTTACGCCACCAGCTCCAAACGG-$(CH_2)_7$-NH-MB-3'

The conventional E-DNA sensor requires a probe with a direct thiol modification at the 5'-end and the sequence is as follows:

5' HS-$(CH_2)_{11}$-CCGTTACGCCACCAGCTCCAAACGG-$(CH_2)_7$-NH-MB-3'

The *K-ras* is a gene that encodes one of the proteins in the epidermal growth factor receptor (EGFR) signaling pathway. Pancreatic and lung cancers harbor high incidences of *K-ras* mutant alleles, and these mutations are early events in colorectal tumor development.[1] The detection of *K-ras* mutations enables understanding of cancer biology and pathogenesis.

The target DNA sequence (WT-Gly) was obtained via commercial synthesis (polyacrylamide gel electrophoresis purification, Integrated DNA Technologies, Coralville, IA), and the sequence is as follows:

WT-Gly: 5'-TTGGAGCTGGTGGCG TA-3'

Electrochemical measurements were performed at room temperature (22±1°C) using a CHI 1040A Electrochemical Workstation (CH Instruments, Austin, TX). Polycrystalline gold disk electrodes (CH Instruments, Austin, TX) with geometric area of 0.0314 cm^2 were used as working electrodes. The counter electrode used was a platinum wire electrode and a Ag/AgCl (3M 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.

Preparation of a "Click" Chemistry-based E-DNA Sensor. Prior to sensor fabrication, azide-terminated undecyl disulfide was first reduced to 1-azidoundecan-11-thiol (C11-N_3) in presence of TCEP. A cleaned gold electrode was placed in a 200 μM solution of C11-N_3 prepared in 3:1 EtOH/H_2O for 10 min. The electrode was subsequently incubated in a 2 mM C11-OH solution in 4:1 EtOH/H_2O for ~2.5 hrs to complete the monolayer formation. The mixed monolyaer-modified elctrode was then rinsed with copious amount of DI water, dried with N_2 gas, and transferred to a "click" mixture containing 900 μM TBTA, 400 μM sodium ascorbate, 400 μM $CuSO_4$ and 3.5 - 4 μM of alkyne-modified

probe DNA in 1:1 DMSO/H_2O. The "click" reaction was allowed to proceed for 30 min in dark. To remove physically adsorbed probe DNA, the electrode was rinsed thoroughly with DI water, 5% tween, EtOH and then again in the reverse order. Prior to transferring the sensor electrode to the electrochemical cell for target interrogation, the electrode was rinsed with DI water for 30 sec.

Two additional sensors were fabricated to evaluate the extent of non-specific adsorption of probe DNA during the "click" reaction. The two sensors were fabricated following the above procedures, with the exception that one sensor was fabricated in absence of $CuSO_4$, whereas the other sensor was constructed without sodium ascorbate, the reducing agent.

Preparation of a Conventional E-DNA Sensor. Prior to sensor fabrication, the probe DNA was first reduced to its free thiol form in presence of TCEP. A cleaned gold electrode was first immersed in a 2 mM C11-OH ethanolic solution for 5 min. The electrode was rinsed with EtOH and DI water, and subsequently immersed in a Phys2 buffer containing 2 μM probe DNA for 2 hr. The sensor electrode was treated with a 30-sec DI water rinse prior to introduction to the electrochemical cell for target interrogation.

Electrochemical Measurements. E-DNA sensor performance was analyzed by alternating current voltammetry (ACV). Alternating current (AC) voltammograms were recorded in a Phys2 buffer or 100% human whole blood from 0 V to -0.5 V vs. Ag/AgCl with a 10 Hz, 25 mV AC potential. Prior to target interrogation, the electrodes were allowed to equilibrate in a Phys2 buffer for at least 20 minutes. The E-DNA sensor response to the target DNA was measured by incubating the electrodes in 1 μM of the WT-Gly target DNA (in a Phys2 buffer or human whole blood). The sensors were interrogated at different intervals in the target solution until a stable peak current was obtained. 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.

Sensor regeneration was achieved by rinsing for 30 sec with deionized water for sensors utilized in Phys2 buffer or by incubating with 4 M GHCl for 4 min, followed by rinsing with deionized water for 30 sec for sensors utilized in whole blood. Sensor regeneration

was verified by AC voltammogram recorded 5 minutes after re-immersion in the buffer or whole blood.

The number of electroactive DNA probes on the electrode surface, N_{tot} was determined using a previously established relationship with ACV peak current described in eq. 1:[2, 3]

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

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 DNA probes was measured in the number of electroactive DNA probes per unit area.

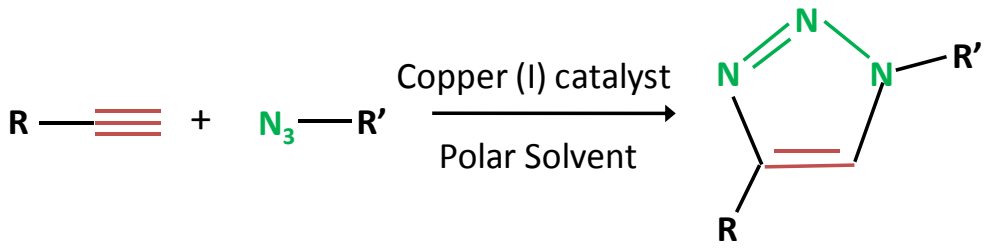
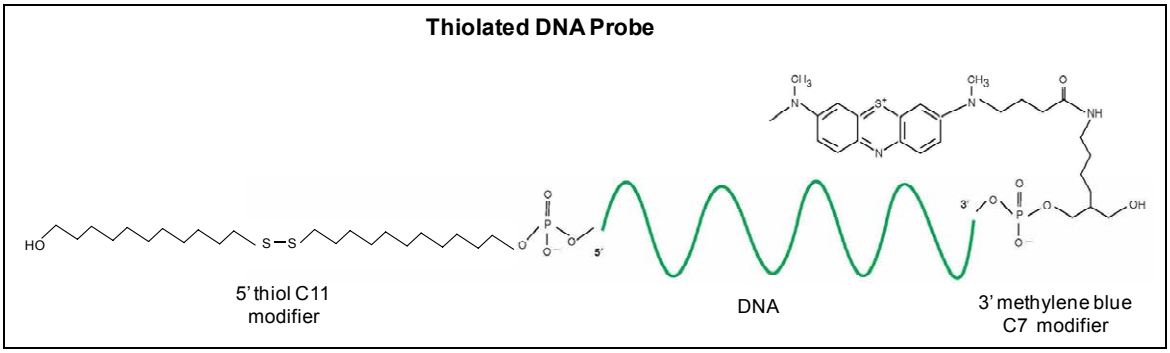


Fig. SI1 Sharpless "click" chemistry.

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

R = C3-DNA-C7-methylene blue

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



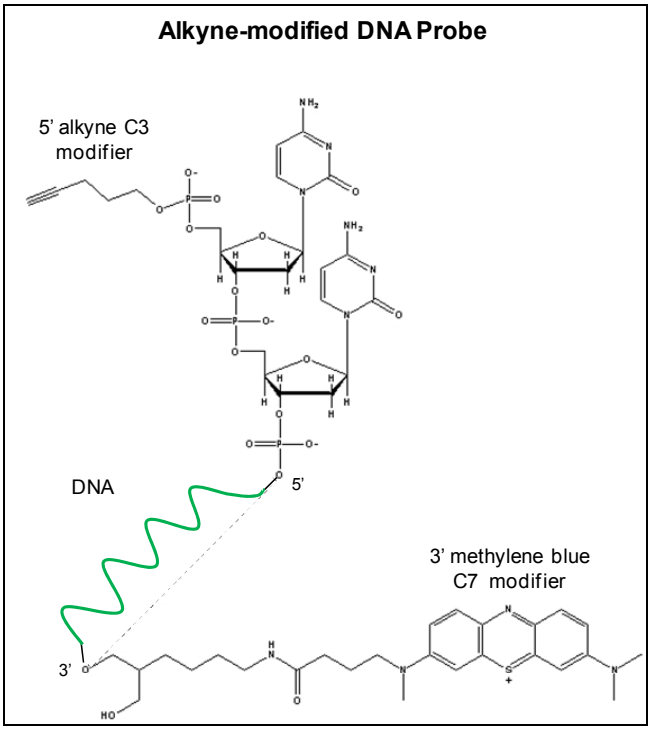


Fig. SI2 Structures of the probe DNA used in the conventional (top) and "click" chemistry-based (bottom) E-DNA sensors.

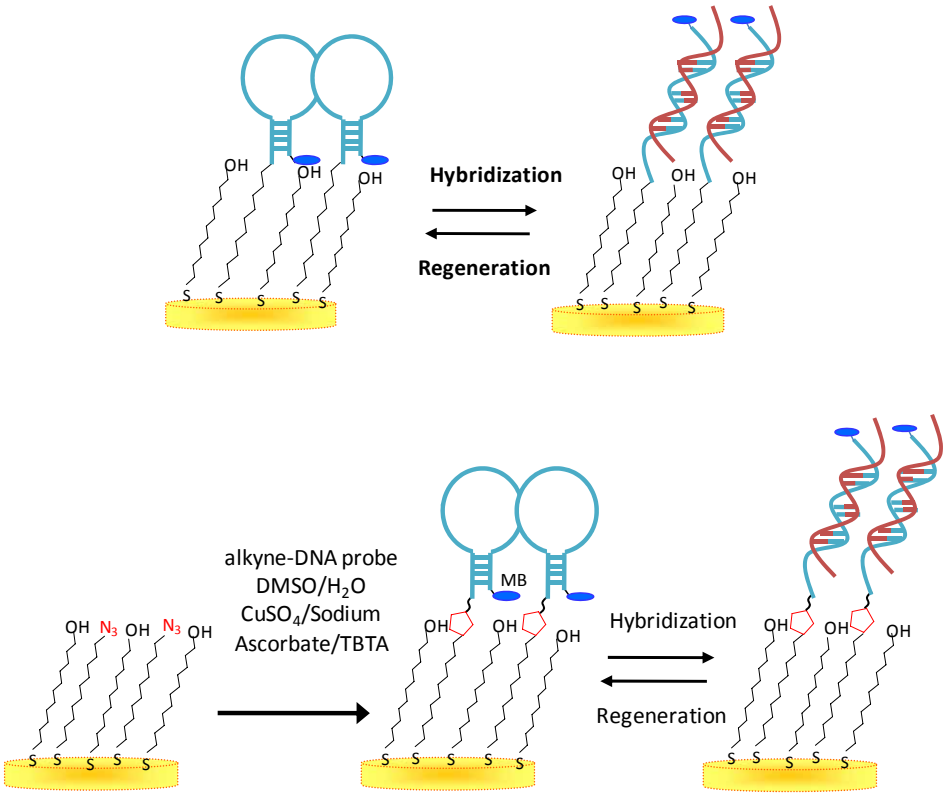


Fig. SI3 The conventional (top) and "click" chemistry-based (bottom) E-DNA sensor constructs.

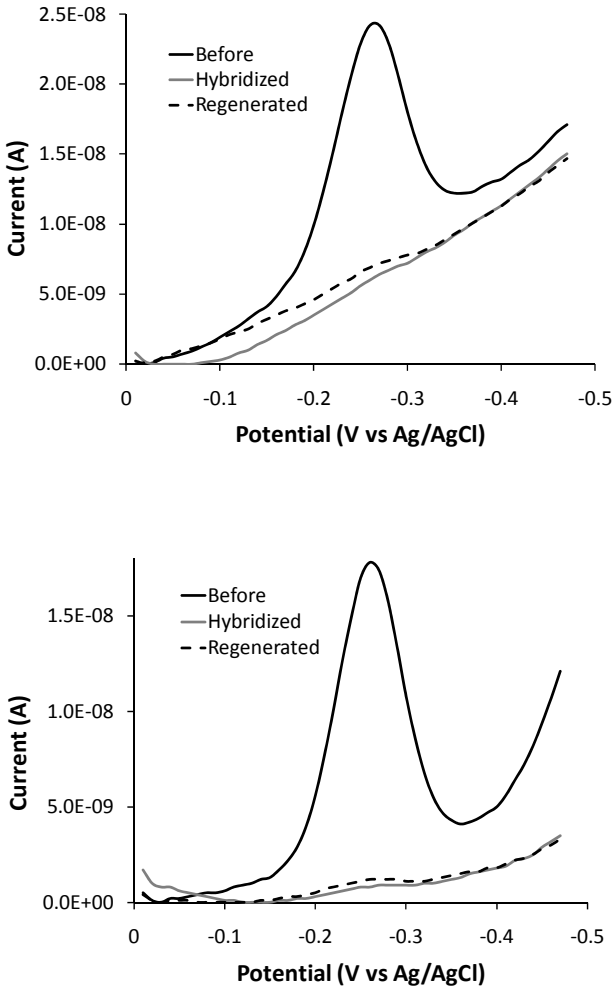


Fig. SI4 AC voltammograms of "click" chemistry-based E-DNA sensors fabricated in absence of copper sulfate (top) and sodium ascorbate (bottom) before, after incubation with 1 μM of target DNA, and after regeneration via a 30-sec room temperature DI water rinse. Since sensor regeneration is a unique property of the E-DNA sensor, it can be used as a gauge for sensor performance. Sensors with physically adsorbed DNA probes cannot be regenerated since the probes will be desorbed by successive voltammetric scans and DI water rinses.

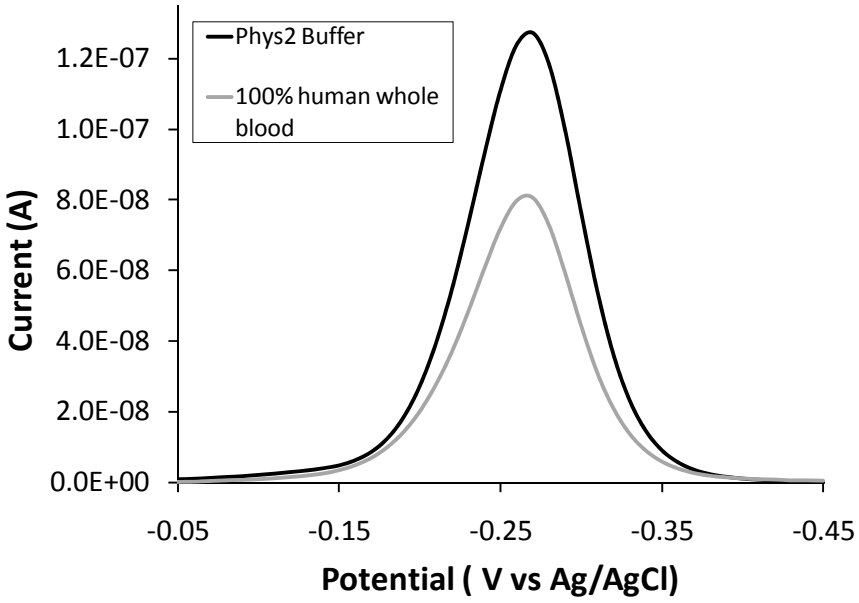


Fig. SI5 E-DNA sensor response to human whole blood. A ~37% signal reduction is evident when the sensor was transferred from a Phys2 buffer to the whole blood matrix. The reduction in current is probably due to the differences in the viscosity and ionic strength, which is known to affect the MB current in E-DNA sensors.

References

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