Supporting Information

Folding-based electrochemical DNA sensor fabricated on a gold-plated screen-printed carbon electrode†

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MATERIALS AND METHODS

Materials and Instrumentation. The reagents 6-mercapto-1-hexanol (C6-OH), hydrogen tetrachloroaurate hydrate ($HAuCl_4 \cdot 3H_2O$), 8 M guanidine hydrochloride, tris-(2-carboxyethyl) phosphine hydrochloride (TCEP), trizma base and iron-supplemented fetal calf serum were used as received (Sigma-Adrich, St. Louis, MO). All other chemicals were of analytical grade. All the solutions were made with deionized water purified through a Milli-Q system (18.2 MΩ·cm, Millipore, Bedford, MA). Physiological buffer solution (Phys, pH 7.0) consisted of 20 mM Tris, 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ and 1 mM $CaCl_2$.

A thiol and methylene blue (MB)-modified stem-loop oligonucleotide complementary to the *K-ras* gene was used as probe DNA (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 (MB-NHS, EMP Biotech, Berlin) producing the probe sequence 5' HS-$(CH_2)_6$-CCGTT*ACGCCACCAGCTCCA*AACGG-$(CH_2)_7$-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 its sequence was 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). The screen-printed carbon electrodes (SPCE) (Pine Instrument, Grove City, PA) were used as the substrates of the sensor, which consisted of a 2 mm diameter screen-printed carbon working electrode, a screen-printed silver-chloride reference electrode and a screen-printed counter electrode. Scanning electron microscopy (SEM) analysis was performed on a Hitachi S-4700 field-emission SEM (Hitachi High Technologies America, Inc. Schaumburg, Illinois) at an acceleration voltage of either 10 kV or 20 kV.

E-DNA Sensor Preparation. SPCE were used as substrates to fabricate the E-DNA sensors. A gold film was electrodeposited on SPCE by holding the SPCE at -0.40 V vs. Ag/AgCl (3 M KCl, external refrence electrode) in a stirred gold solution (1.2 mg mL^{-1} $HAuCl_4$, 1.5 wt. % HCl and 0.1 M NaCl) for various depostion time to produce a gold-plated screen-printed carbon electrode (GPE). The GPE were 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 the electrode was determined from the charge associated with the gold oxide stripping peak obtained after the cleaning process.

For the fabrication of E-DNA sensors, a mixture of 1 μL of the 200 μM probe DNA with 1 μL of 10 mM TCEP was first incubated for 1 h to reduce the disulfide bond of the probe DNA, followed by diluting the solution to 100 μL with Phys. The probe DNA was immobilized onto the surface of GPE by incubating the clean electrodes in the diluted probe DNA solution (2 μM DNA) for 1 h. The electrodes was then rinsed with water and subsequently passivated with 2 mM 6-mercapto-1-hexanol (C6-OH) for 2 h to displace nonspecifically bound oligonucleotides.

Electrochemical Measurements. E-DNA sensor measurements were traditionally conducted using alternating current voltammetry (ACV) over the range -0.15 V to -0.55 V with a frequency of 10 Hz and an amplitude of 25 mV. Prior to interrogation the electrodes were allowed to equilibrate in Phys for 30 min. The E-DNA sensor response was measured by incubating the electrodes in 1 μM of the WT-Gly target DNA (in Phys or in undiluted fetal calf serum). 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 s with deionized water for sensors utilized in Phys or by incubating with 4 M guanidine-HCl for 4 min, followed by rinsing with deionized water for 30 s for sensors utilized in serum.

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]

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

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. Perfect transfer efficiency was assumed, and errors in this assumption would lead us to underestimate probe density. Experimentally, three different frequencies were used (5, 10, 20 Hz), and the average peak current was calculated so as to give the value of N_{tot}. The surface density of DNA probes was measured in the number of electroactive DNA probes per unit gold area.

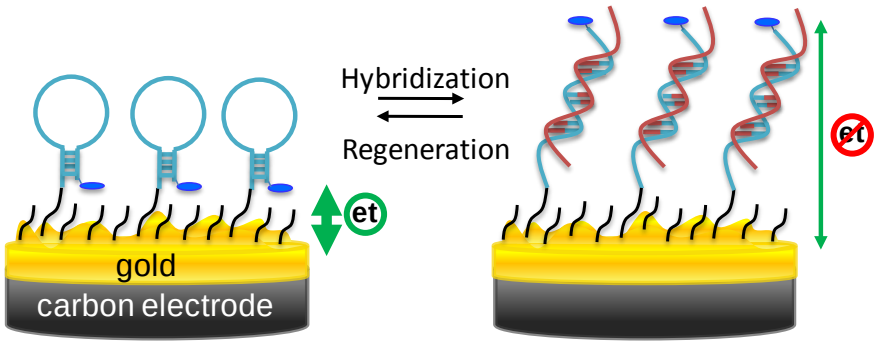

Fig. SI1 A GPE-based E-DNA sensor construct and signaling mechanism.

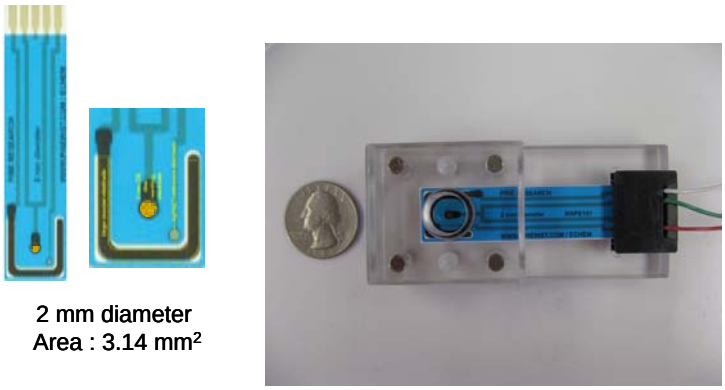

Fig. SI2 The gold-plated screen-printed carbon electrode (GPE) and the sensor device.

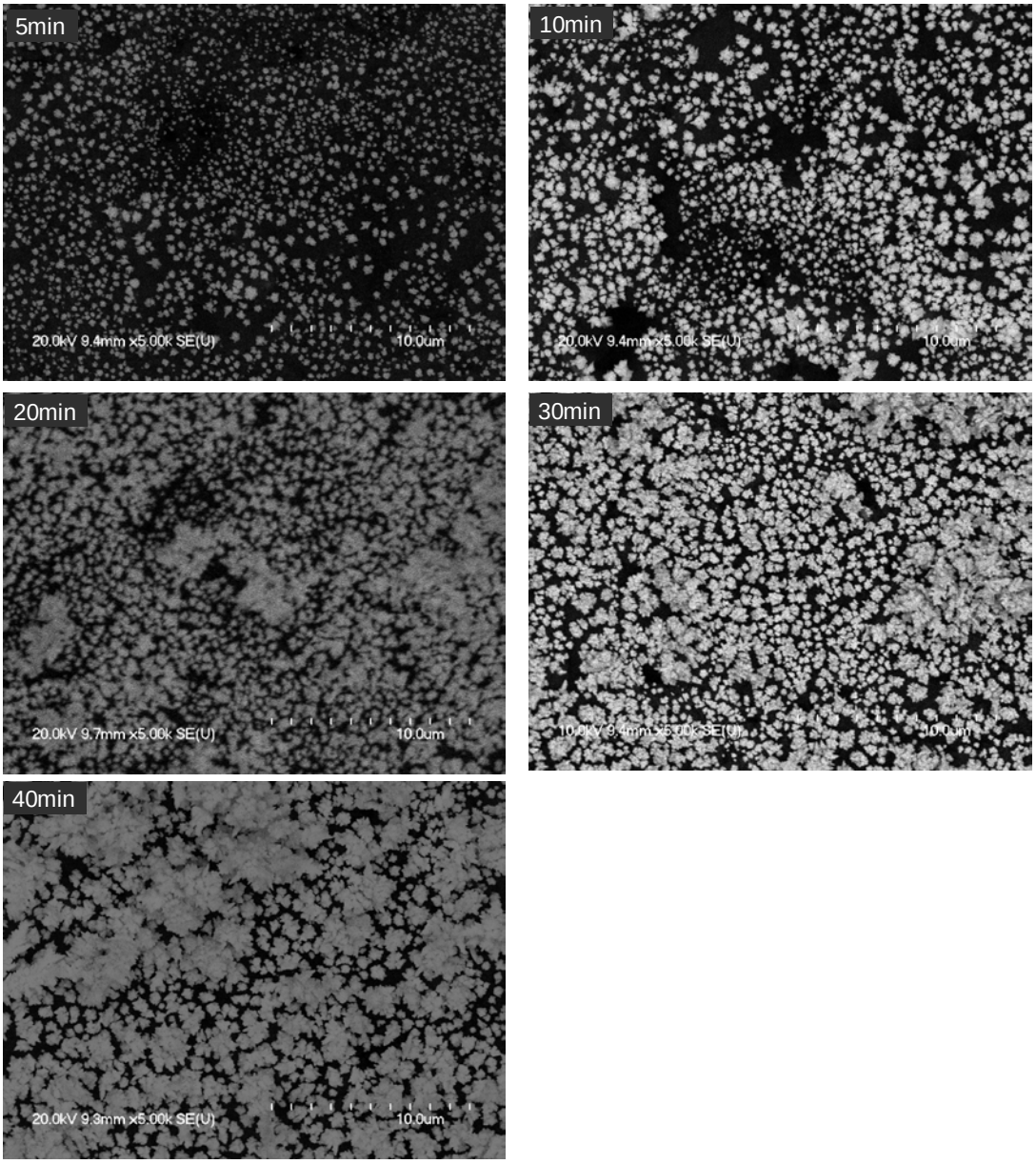

Fig. SI3 SEM images of GPEs with varied deposition time. Conditions: direct current voltage of -0.40 V (*vs.* Ag/AgCl) in stirred gold solutions (1.2 mg mL^{-1} $HAuCl_4$, 1.5 wt. % HCl and 0.1 M NaCl); Top: left to right: 5 min, 10 min; Center: left to right: 20 min, 30 min; Bottom: 40min.

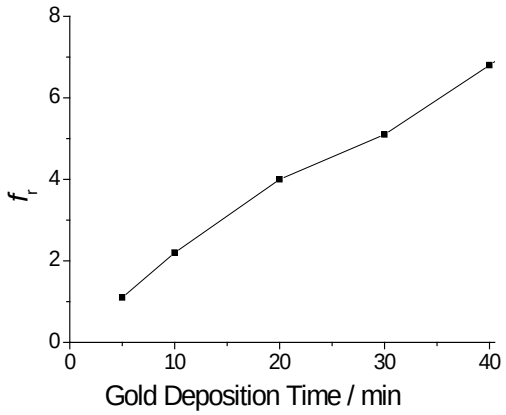

Fig. SI4 Influence of gold deposition time on the roughness factor (f_r) of the GPE surface.

References

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