Supporting Information

Experimental

Materials. All DNA oligos were purchased from Sangon Inc. (Shanghai, China). The capture probe is a thiolated oligo with the sequence of 5'-HS-C_6-ATGATGTTCGTTGTGTAGGATTTGC-3'; its target (complementary DNA) has a sequence of 5'-GCAAATCCTACACAACGAACATCAT-3'. Tris-(hydroxymethyl)aminomethane was from Cxbio Biotechnology Ltd. Ethylenediaminetetraacetic acid (EDTA), potassium ferricyanide ($K_3Fe(CN)_6$), mercaptohexanol (MCH), hexaammineruthenium (III) chloride ($[Ru(NH_3)_6]^{3+}$, RuHex) and tris (2-carboxyethyl) phosphine hydrochloride (TCEP) were from Sigma. Unless otherwise indicated, all reagents were used without further purification. The buffer solutions involved in this study are as follows. DNA immobilization buffer (IB): 10 mM Tris-HCl, 1 mM EDTA and 10 mM TCEP (pH 7.6). TCEP was used to cleave disulfides in thiolated DNA. Hybridization buffer (HB): 10 mM Tris-HCl, 1 mM EDTA and 1 M NaCl (pH 7.6). Buffers for both electrode washing and electrochemical measurements were 10 mM Tris-HCl solutions (pH 7.4). All solutions were prepared with MilliQ water (18 MΩ·cm) from a Millipore system.

Electrochemical Measurements. All electrochemical measurements were performed with a CH 650 electrochemical workstation (CH Instruments Inc., Austin). A conventional three-electrode configuration was employed all through the experiment, which involved a gold working electrode, a platinum wire auxiliary electrode, and an Ag/AgCl reference electrode. Cyclic voltammetry (CV) was carried out at a scan rate of 50 mV/s, and chronocoulometry (CC) at a pulse period of 250 ms and pulse width of 700 mV. The electrolyte buffer was thoroughly purged with nitrogen before experiments.

DNA self-assembly and hybridization at gold electrodes. Gold electrodes (2 mm in diameter, CH Instruments Inc., Austin) were first polished on microcloth (Buehler) with Gamma micropolish deagglomerated alumina suspension (0.05 μm) for 5 min. Residual alumina powder were removed by sonicating electrodes in ethanol and water for 5 min, respectively. Then the electrodes were electrochemically cleaned to remove any remaining impurities.[1, 2] After being dried with nitrogen, electrodes were immediately used for DNA immobilization.

Electrodes with DNA self-assembly monolayers (SAMs) of different surface density were interrogated in this work. Low-density surfaces (2.5 pmol/cm^2) were obtained by incubation of electrodes with 0.2 μM of capture probes in the IB with 0.1 M NaCl for 30 min. Medium-density (5.2 pmol/cm^2, 6.5 pmol/cm^2), and high-density (20 pmol/cm^2) surfaces were prepared by incubation of electrodes with 0.5 μM, 1.0 μM and 5.0 μM of capture probes in the IB with 1 M NaCl for 60 min, respectively. Thus prepared DNA modified electrodes were further treated with 1 mM MCH for 2 h in order to obtain well-aligned DNA monolayers.

DNA hybridization was performed by incubation of electrodes at 0.1 μM solution of target oligonucleotide in HB for 60 min at 37 °C. After hybridization, electrodes were extensively rinsed with washing buffer and dried under a stream of nitrogen prior to characterization.

Quantitation of surface density of probes. The surface density of ssDNA can be calculated using the assumption that RuHex molecule stoichiometrically binds to the anionic phosphodiester backbone of DNA.[3] When an electrode modified with DNA is placed in a low ionic strength electrolyte containing a mulitivalent redox cation, the redox cation exchanges with the native charge compensation cation and becomes electrostatically trapped at that interface. The surface densities of DNA probes can then be calculated from the redox charges of RuHex according to the chronocoulometric methods first proposed by Tarlov and co-workers.[4] It can be easily calculated from

$$\Gamma_{DNA}=(QN_A/nFA)\cdot(z/m)$$

where **n** is the number of electrons in the reaction, **A** is the area of the working electrode, **m** is the number of nucleotides in the DNA, **z** is the charge of the redox molecules, and $\mathbf{N_A}$ is Avogadro's number. **Q** is the charge which can be obtained either by integrating of the redox peaks in the cyclic voltammograms or by calculating the chronocoulometric intercept at t = 0. Chronocoulometry provides an accurate measure of the surface excess of redox marker at a DNA modified electrode, which was then employed in the following quantification experiments.[4, 5]

Electrocatalytic Detection of Target Hybridization. The electrocatalytic current obtained at gold electrodes modified with thiolated probe DNA was measured in buffers containing 10 μM RuHex and 2

mM $K_3Fe(CN)_6$. Hybridization was detected through electrocatalysis-associated enhancement of the reduction currents.

References

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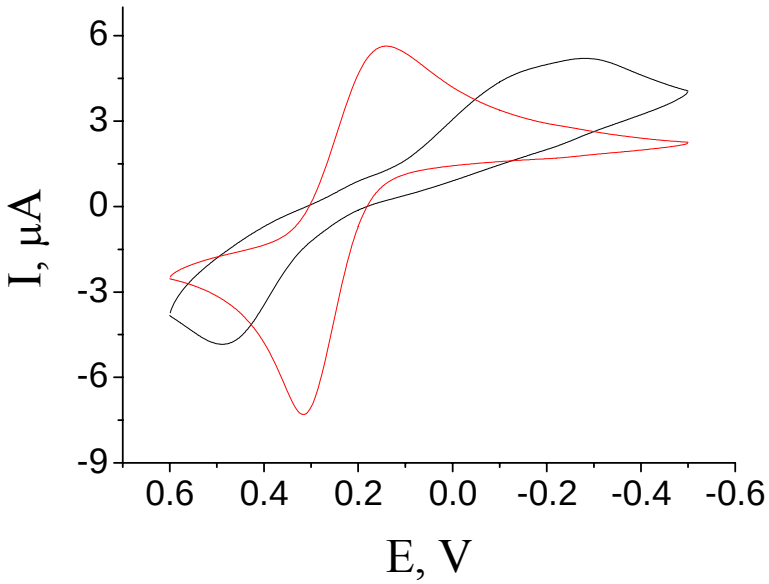

Figure 1-S. CVs of gold electrodes modified with A) HD (20 pmol/cm^2, black) and B) LD (2.5 pmol/cm^2, red) SH-DNA in 10 mM Tris buffer (pH 7.4) with 2 mM $K_3Fe(CN)_6$. Scan rate: 50 mV/s. Of note, the positive current after 0 V (at the LD surface) is responsible for the upward shift of CVs in Figure 1B.

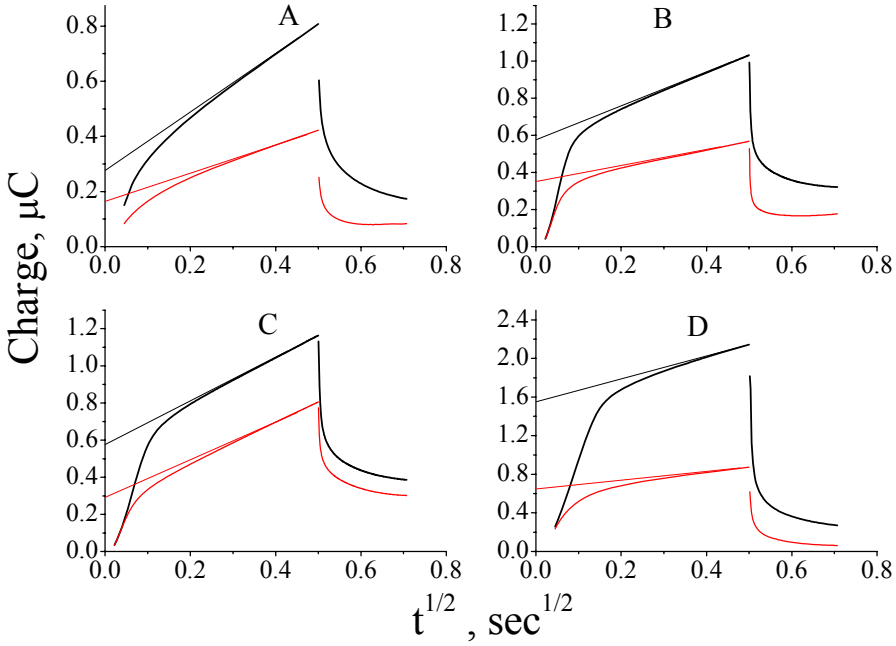

Figure 2-S. Representative chronocoulometric curves for gold electrodes modified with A) 2.5 pmol/cm^2 B) 5.2 pmol/cm^2 C) 6.5 pmol/cm^2 and D) 20.0 pmol/cm^2 SH-DNA probes in 10 mM Tris buffer (pH 7.4) (red lines) and with 50 µM RuHex (black lines). Redox charges of RuHex bound to DNA are obtained from chronocoulometric intercepts at t = 0. The signal is defined as the increment of the redox charge, i.e., Signal = Q_{ru}-Q_{bl}.