

**Supporting Information for:**

# Determinants of the Detection Limit and Specificity of Surface-Based Biosensors

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### *Reagents and DNA probes*

Reagent grade chemicals, including sodium chloride (NaCl), trizma hydrochloride ( $C_4H_{11}NO_3 \cdot HCl$ ), ethylenediaminetetraacetic acid (EDTA), 6-mercapto-1hexanol ( $C_6-OH$ ), tris-(2-carboxyethyl) phosphine hydrochloride (TCEP), hexaammineruthenium (III) chloride ( $Ru(NH_3)_6Cl_3$ ) (all from Sigma-Aldrich), sodium phosphate monobasic ( $NaH_2PO_4 \cdot H_2O$ ) and sodium phosphate dibasic anhydrous ( $Na_2HPO_4$ ) (all from Carlo Erba Reagenti) were used without further purification. A 27-base 5' thiol-, 3' methylene blue (MB)-modified probe DNA (5'-d Thiol C6 SS-CGT CAA TCT TCT ATT TCT CCA CAC TGC T-methylene blue-3') was obtained from Biosearch (Novato, CA), and employed as the probe DNA.

### *Target DNA sequences*

We employed the following target DNA sequences of varying lengths and structures, all of which were obtained via commercial synthesis (Sigma-Aldrich). The target sequences were as follows:

**T-27** (27-base target, 5'-GCA GTG TGG AGA AAT AGA AGA TTG ACG-3'); **MM-27** (27-base mismatch target, 27 bases, 5'-GCA GTG TGG ACA AAT AGA AGA TTG ACG-3'); **T-22** (22-base target, 5'-GGA GTG TGG AGA AAT AGA AGA T-3'); **MM-22** (22-base mismatch target, 5'-GCA GTG TGG AGA AAT AGA AGA T-3'); **T-17** (17-base target, 5'-GTG GAG AAA TAG AAG AT-3'); **MM-17** (17-base mismatch target, 5'-GTG GAC AAA TAG AAG AT-3'); **T-14** (14-base target, 5'-TGG AGA AAT AGA AG-3'); **MM-14** (14-base mismatch target, 5'-TGG ACA AAT AGA AG-3'); **T-13** (13-base target, 5'-GGA GAA ATA GAA G-3') and **MM-13** (13-base mismatch target, 5'-GGA CAA ATA GAA G-3').

In the above sequences underlined bases identify the mismatched bases.

### *Sensor Fabrication*

E-DNA sensors were manufactured on 3 mm diameter gold screen printed-electrodes<sup>14</sup>, fabricated as previously described<sup>2</sup> and used without any pretreatment. The first step in sensor fabrication is

the reduction of the probe DNA (200  $\mu$ M) for 1 h in a solution of 10 mM tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) prepared in 1 M NaCl/10 mM potassium phosphate buffer, pH 7.0. Then, the modified electrode surface was covered with a 20  $\mu$ L drop of the respective probe solution (prepared in 150 mM NaCl/50 mM potassium phosphate buffer, pH 7.0) incubated for 1 h and rinsed with distilled, deionized water to remove the excess of DNA probe. In order to displace nonspecifically adsorbed DNA and passivate the remaining electrode area, was subsequently incubated for 1.5 h in a 20  $\mu$ L drop of 2 mM 6-mercaptohexanol solution (also prepared in 150 mM NaCl/50 mM potassium phosphate buffer, pH 7.0), followed by further rinsing with deionized water. Finally, the electrodes were stored in the phosphate buffer solution for 30 min before their use.

#### *Electrochemical Measurements and binding curves*

The sensors were interrogated at intervals of 5 min in the buffer solution until a stable current peak was obtained (typically after 30 min). Then, the sensor response was measured by incubating the electrodes for 1 h in the appropriate target DNA concentration. The ratio between the stabilized current peak in the presence of target DNA and the current peak in the absence of target DNA gives the measure of the signal suppression caused by the target. All experiments were performed using a PalmSens potentiostat instrument using a CH8 multiplexer that allows the measurement of eight different screen printed electrodes at the same time. Square wave voltammetry (SWV) was recorded from -0.4 V to -0.05 V versus Ag/AgCl reference electrode using a 25 mV potential at a frequency of 50 Hz. All experiments were conducted in 150 mM NaCl/50 mM potassium phosphate buffer, pH 7.0. Binding curves were obtained by interrogating each sensor in buffer solution to obtain the background signal and then at different target concentrations until signal suppression was stable. The actual signal changes have been normalized on a 0-100 scale to provide more clear interpretation of the results.

### *Calculation of Probe Surface Density*

Probe surface-density (the number of electroactive probe DNA moles per unit area of the electrode surface) was determined using a previously established method<sup>15</sup>. In brief, the measured charge acquired from the reduction of the RuHex that is electrostatically associated with the negatively charged backbone of the surface-bound probes is used to calculate the number of moles of phosphate (and thus number of probe DNAs) immobilized on the surface. More specifically, chronocoulometric experiments were acquired using 10 mM Tris buffer (pH 7.4) or 0.2 mM RuHex in 10 mM Tris buffer (pH 7.4). Electrodes were immersed in the respective solution for 10 min prior to the experiment. Two-step coulometry was performed stepping from 0.1 to -0.4 V versus Ag/AgCl with a pulse period of 250 ms and a sample time of 0.001 s. The surface excess of RuHex at a DNA-modified electrode is determined from the difference in intercepts for the response in the absence and presence of redox marker<sup>15</sup>.

The density with which probe are packed on the electrode surface is controlled by varying the concentration of thiolated oligonucleotide employed during sensor fabrication. Employing fabrication concentrations of 0.01–2  $\mu\text{M}$  we reproducibly achieve probe densities ranging from  $0.37 \times 10^{-12}$  to  $3.23 \times 10^{-11}$  moles/cm<sup>2</sup>.