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Supporting Information

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Re-engineering Electrochemical Biosensors To Narrow or Extend Their Useful Dynamic Range**

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Supporting Information:

Materials and Methods

The following reagents were used as received: sodium phosphate monobasic (Sigma Aldrich), sodium phosphate dibasic heptahydrate (Sigma Aldrich), sodium chloride (Sigma Aldrich), tris(2-carboxyethyl)phosphine hydrochloride (TCEP) (Molecular Probes, Carlsbad, CA).

Signalling probes, depletants and target sequences were commercially sourced (Biosearch Technologies, Novato, CA) and used as received. Their sequences were as follows:

Linear Probe, 5'- HS-$(CH_2)_6$- CGTCA GATCGGCGTTTTA ACTGC-$(CH_2)_7$-NH-MB-3′

Probe 0GC, 5'- HS-$(CH_2)_6$-*ATTATT* GATCGGCGTTTTA *AAGAAG*--$(CH_2)_7$-NH-MB-3′

Probe 1GC, 5'- HS-$(CH_2)_6$-*ACTATT* GATCGGCGTTTTA *AATAGG*--$(CH_2)_7$-NH-MB-3′

Probe 3GC, 5'- HS-$(CH_2)_6$-*ACTCTC* GATCGGCGTTTTA *GAGAGG*--$(CH_2)_7$-NH-MB-3′

Thiol modified depletant, 5'- HS-$(CH_2)_6$-ATTATT GATCGGCGTTTTA-3′

Depletant, 5' – CGTCA GATCGGCGTTTTA ACTGC - 3′

Target: 5′-TAAAACTCCGATC-3′.

Where -$(CH_2)_7$-NH-MB-3′ represents a methylene blue (MB) added to the terminal phosphate via a C-7 amino linker and 5'- HS-$(CH_2)_6$ represents a thiol group used for attachment to the electrode surface. In the sequences above the recognition portion is underlined, while the stem portion is in italics. Of note, linear probe and depletant do not form a stem-loop structure as their stems sequences are random and not self-complementary.

Electrode Preparation and Sensor Fabrication

E-DNA sensors were prepared using a well-established previously described procedure [1]. In brief, prior to sensor fabrication, gold disk electrodes (2 mm diameter, CH Instruments, Austin, TX) or screen printed electrodes[2] were cleaned both mechanically and electrochemically. Before use, probe and, when needed, thiol-modified depletant were reduced for 1 hr at room temperature in the dark in 10 mM TCEP. The relevant DNA probes were then immobilized onto freshly cleaned electrodes by incubating for 1 hr in 500 mM NaCl/10 mM potassium phosphate, pH 7 buffer. To achieve a wide dynamic range, the probe 0GC and 3GC were mixed in a 1:1 ratio at a total concentration of 1 μM onto classic rod electrodes. To narrow the dynamic range of the E-DNA sensors we employed two different strategies. In the first strategy we used a mixture of immobilized signalling probe (1GC) and the thiol-modified depletant using classic rod electrodes. To achieve different probe/depletant ratios on the electrode surface, the electrodes were

immersed in a mixture of the probe 1GC and the thiol-modified depletant at varying concentration ratios of 1:1, 1:10, 1:25, 1:50 and 1:100 with a total concentration of 1 μM. The electrodes were then rinsed with distilled, deionized water, and incubated in 3 mM 6-mercapto-1-hexanol in 500 mM NaCl/10 mM potassium phosphate, pH 7 buffer for 30 min. In a second strategy we used a linear signalling probe immobilized onto the surface of a gold screen printed electrode[3] and a silent depletant free in solution at different concentrations to achieve different threshold response. Of note, in this case the depletant is not modified with the thiol group because no immobilization to the electrode is needed. The electrodes were immersed in a mixture of the linear probe at a concentration of 1 μM. The electrodes were then rinsed with distilled, deionized water, and incubated in 3 mM 6-mercapto-1-hexanol in 500 mM NaCl/10 mM potassium phosphate, pH 7 buffer for 30 min. Following both these preparation procedures, the electrodes were rinsed in water and stored in buffer for future use.

Electrochemical Measurements

Fabricated sensors were interrogated using square wave voltammetry (SWV) with a 50 mV amplitude signal at a frequency of 60 Hz, before and after a hybridization step with increasing concentrations of the complementary target. To do this, the electrodes were first interrogated in a pure buffer solution (background signal) 1 M NaCl/10 mM potassium phosphate, pH 7 and then incubated for 30 min in a solution with the appropriate concentration of target DNA. After this incubation, the electrodes were transferred back to the blank buffer solution for a new SWV measurement. Signal gain was computed by the relative change in SWV peak currents with respect to background current. When attempting to achieve a narrow range we have employed two different strategies. In the first strategy we have employed a mixture of a signalling probe (1GC) and a depletant both immobilized on the electrode surface. For this strategy the volume of the hybridization solution was kept at a minimum allowable of 3 μL. In fact, it should be considered that since the depletant is immobilized on the electrode, its amount is fixed and determined by its surface density. The threshold value at which the sensor responds will therefore depend on the volume of the hybridization solution. And since the surface density of a sensor has also a maximal limit, this limits the total amount of depletant available for hybridization with the target and thus could explain the plateau in sensitivity that we observe in our experiments. In addition we note that the ratio of the depletant concentration to the probe concentration employed during sensor fabrication cannot exceed 100; at higher ratios the density of probes on the surface is so low that the electrochemical signal degrades (Figure SI3), resulting in unacceptable sensor-to-sensor variability.[3] In the second strategy, instead, the depletant is free in solution and there are no limitations with regards to hybridization volume. Values and graphs with reported error bars represent the average and standard deviations of measurements performed on at least three independently fabricated electrodes.

References:

[1] a) Y. Xiao, R. Y. Lai, K. W. Plaxco, *Nat. Protoc.* **2007**, *2*, 2875-2880; b) A. A. Rowe, R. J. White, A. J. Bonham, K. W. Plaxco, *J. Vis. Exp.* **2011**, e2922; c) D. Kang, X. L. Zuo, R. Q. Yang, F. Xia, K. W. Plaxco, R. J. White, *Anal. Chem.* **2009**, *81*, 9109-9113

[2] F. Ricci, G. Adornetto, D. Moscone, K. W. Plaxco, G. Palleschi, *Chem. Comm.* **2010***, 46*(10), 1742-1744

[3] F. Ricci, R. Y. Lai, A. J. Heeger, K. W. Plaxco, J. J. Sumner, *Langmuir* **2007**, *23*, 6827-6834

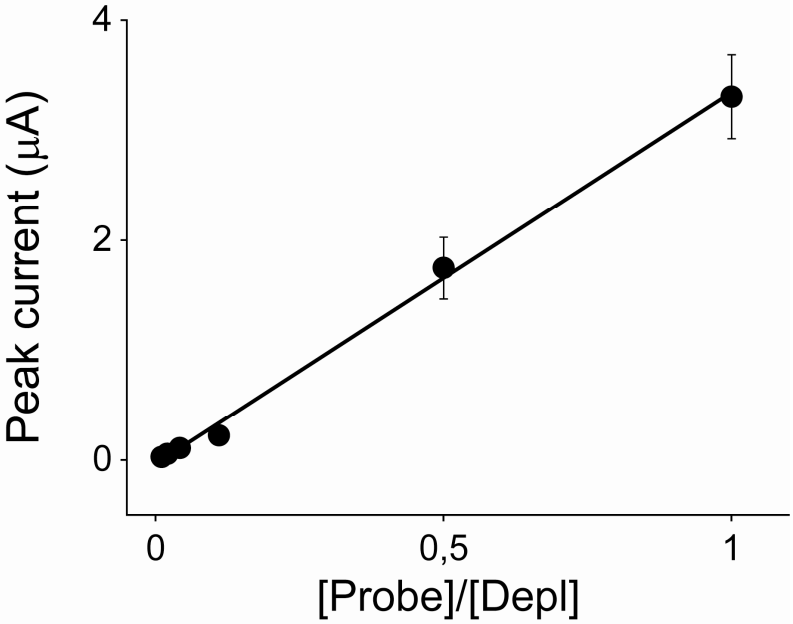

Figure SI1: The amount of signalling probe immobilized on the surface of our sensors (which is linearly correlated with the peak current observed with SWV [3]) varies linearly with the ratio of [signalling probe] to [depletant] presents in solution during fabrication. This seems to confirm that the density ratios of probe and depletant on surface are linearly correlated with the concentration ratios deployed in solution during deposition. We note, however, that this correlation could be more complicated for less defined recognition elements which can induce a non-linear immobilization of probe and depletant (see next figure, Figure SI2)

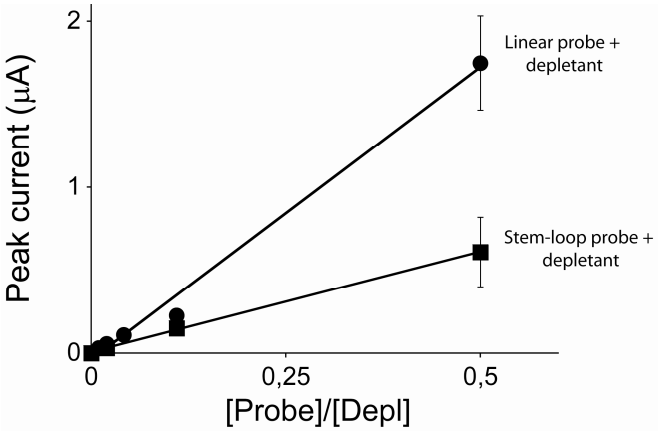

Figure SI2: When signalling probe and depletant are co-immobilized their immobilization rates is dependent on their size and structure. For example, a larger or more complex structured probe deposits less rapidly than a linear probe when coimmobilized with a linear depletant. This demonstrates that the [probe]/[depletant] ratio used during sensor fabrication does not necessarily lead to the same probe/depletant density ratio on surface.

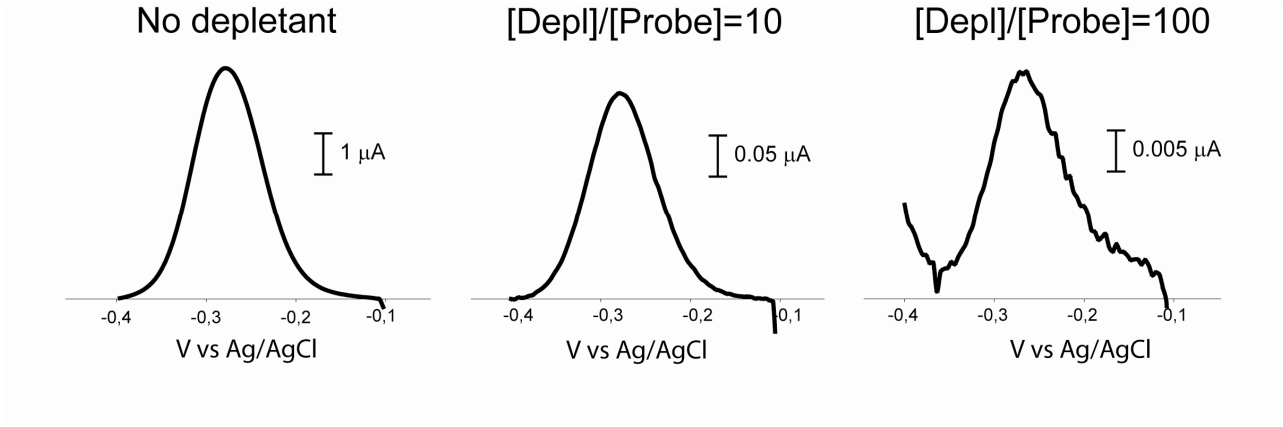

Figure SI3: The first approach proposed here to narrow the dynamic range of a model E-DNA sensor requires the coimmobilization of a signalling probe with a silent depletant. However, this approach is limited to ratio of [depletant]/[probe] used during sensor's fabrication of 100, a value over which signal degradation is so high that it is not possible to record any significant redox signal. Shown are the SWV achieved without depletant (left), and with [depletant]/[probe] ratio used during sensor's fabrication of 10 (center) and 100 (right).