Supporting Information

Aptamer-based Electrochemical Biosensor for Interferon Gamma Detection

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Table of Content.

Experimental Method:

Materials and Reagents: The following reagents were used as received: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sodium bicarbonate ($NaHCO_3$) (all reagent grade),6-mercapto-1-hexanol (MCH), (tris-(2-carboxyethyl) phosphine Hydrochloride (TCEP), and Methylene Blue (MB), Carboxylic Acid, Succinimidyl Ester (MB-NHS, Biosearch Technologies, INC, Novato, CA), Recombinant human IFN-γ was purchased from R&D systems (Minneapolis, MN). The 34-mer IFN-γ-binding aptamer sequence (IDT Technologies, San Diego, CA) was as follows:

5'-NH2-C6- GGGGTTGGTTGTGTTGGGTGTTGTGTCCAACCCC-C3-SH-3'. The aptamer was modified at the 5′-terminus with a C6-disulfide [HO(CH2)6-S-S-(CH2)6-] linker and at the 3′-end with an amine group for redox probe (MB) conjugation. The aptamer was dissolved in 10 mM HEPES buffer (pH 7.4 with 150 mM NaCl). This buffer was also employed in all IFN-γ sensor experiments.

Sensor Fabrication: All sensors were fabricated on gold working electrode (BAS, ∅=1.6 mm) using a procedure similar to that described by Plaxco and coworkers.[1] Briefly, NHS-labeled MB was conjugated to the 3'-end of an amino-modified DNA aptamer through succinimide ester coupling. Prior to modification of the electrodes, aptamer stock solution (0.02 mM) was reduced in 10 mM TCEP for 1 h to cleave disulfide bonds. This solution was then diluted in HEPES buffer to achieve the desired aptamer concentration (from 0.5 μM to 8 μM). For aptamer immobilization, the gold electrodes were kept in solution of thiolated aptamer for 16 h in the dark at 4^{o}C. The electrode surfaces were then passivated by incubating in a 3 mM MCH solution for 1 h.

Electrochemical Measurements: Electrochemical measurements were made using a CHI 842B Electrochemical Workstation (CHInstruments, Austin, TX) with a three electrode system consisting of Ag/AgCl (3 M KCl) reference electrode, Pt wire counter electrode and a gold working electrode. Electrochemical experiments were performed in 10 mM HEPES buffer. Square wave voltammetry was performed using a step potential of 4 mV and a 40 mV amplitude at various frequencies. All sensors were tested before and after incubation for 30min in saturating target concentration (160 ng/mL IFN-γ).

Supporting Information References:

1. Xiao, Y., Lai, R. Y., Plaxco, K. W.; *Nat. Protoc.* **2007**, *2*, 2875-2880.
2. White, R. J., Plaxco, K. W.; *Analytical Chemistry* **2010**, *82*, 73-76.

(A) (B)

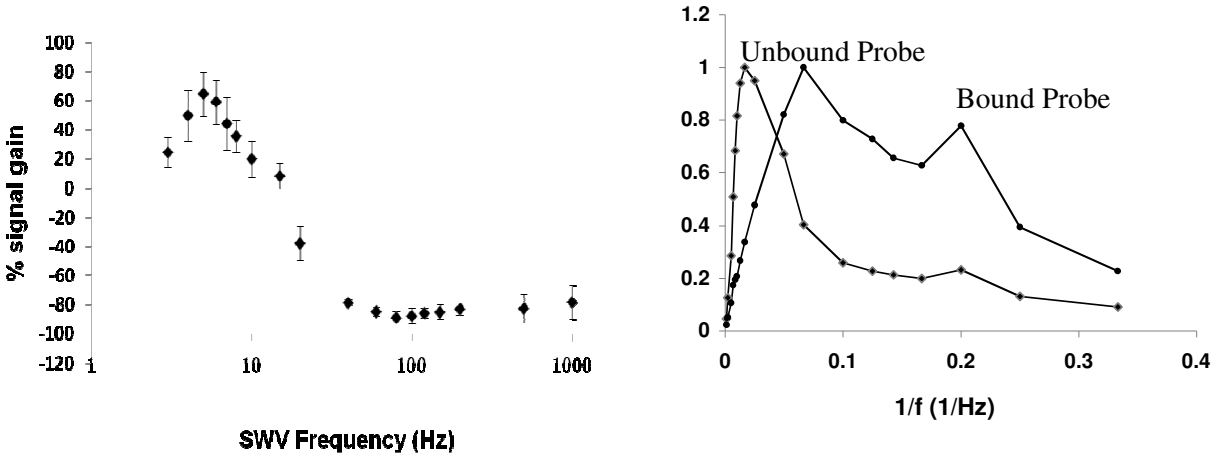

Figure 1: (A) Relationship of signal change and SWV frequency. (B) The relationship between net peak current / f and $1/f$ for unbound probe and bound probe. IFN-γ binding causes a shift to a lower "critical frequency"[2] for our aptamer-based sensor.