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**Rapid and Label-Free Detection of Interferon Gamma via an Electrochemical Aptasensor Comprising a Ternary Surface Monolayer on a Gold Interdigitated Electrode Array**

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**Supporting Information**

**ABSTRACT:** A label-free electrochemical impedance spectroscopy (EIS) aptasensor for rapid detection (<35 min) of interferon-gamma (IFN-γ) was fabricated by immobilizing a RNA aptamer capture probe (ACP), selective to IFN-γ, on a gold interdigitated electrode array (Au IDE). The ACP was modified with a thiol group at the 5′ terminal end and subsequently co-immobilized with 1,6-hexanediol (HDT) and 6-mercaptop-1-hexanolphosphate (MCH) to the gold surface through thiol–gold interactions. This ACP/HDT-MCH ternary surface monolayer facilitates efficient hybridization with IFN-γ and displays high resistance to nonspecific adsorption of nontarget proteins [i.e., fetal bovine serum (FBS) and bovine serum albumin (BSA)]. The Au IDE functionalized with ACP/HDT-MCH was able to measure IFN-γ in actual FBS solution with a linear sensing range from 22.22 pM to 0.11 nM (1–5 ng/mL) and a detection limit of 11.56 pM. The ability to rapidly sense IFN-γ within this sensing range makes the developed electrochemical platform conducive toward in-field disease detection of a variety of diseases including paratuberculosis (i.e., Johne’s Disease). Furthermore, experimental results were numerically validated with an equivalent circuit model that elucidated the effects of the sensing process and the influence of the immobilized ternary monolayer on signal output. This is the first time that ternary surface monolayers have been used to selectively capture/detect IFN-γ on Au IDEs.

**KEYWORDS:** interdigitated electrode array, electrochemical impedance spectroscopy, interferon gamma, aptamer capture probe, ternary surface monolayer, equivalent circuit model

Interferon gamma (IFN-γ) is a strong proinflammatory cytokine with direct antiviral activity, antiproliferative effects, as well as differentiation inducing and immunoregulatory properties. IFN-γ is primarily produced by activated natural killer and T helper cells of the Th1 subset, as well as activated CD8+ cytotoxic cells of the TCI phenotype. IFN-γ is crucially important for generating an effective cell mediated immune response (Th1) to a pathogen, and subsequently elevated levels of IFN-γ can be an early indicator of multiple diseases including tuberculosis, HIV, Crohn’s disease, cancer, and paratuberculosis (i.e., Johne’s disease). The quantification of IFN-γ concentrations in serum and blood samples through various analytical techniques has been explored to further understand the immunological response during disease progression, as well as for the development of rapid diagnostic tools.

Current laboratory methods for detecting IFN-γ are based on an IFN-γ release assay. Here, peripheral blood mononuclear cells are stimulated with appropriate antigens, and the resulting IFN-γ level can be evaluated by a sandwich enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunospot assay (ELISPOT), or reverse transcriptase polymerase chain reaction (RT-PCR). Though these techniques can detect IFN-γ at biologically relevant concentrations (e.g., 5–10 pg/mL for tuberculosis, 15 pg/mL for HIV, and 7.4 pg/mL for Crohn’s disease), they are arduous, expensive, and time-consuming. Furthermore, these techniques, in most cases, require transportation of samples and trained technicians to process the samples.
conduct the diagnostic tests in the laboratory setting—thus, ELISA, ELISPOT, and PCR are generally not amenable to rapid, in-field biodetection.11,12 Besides these conventional laboratory methods, other more rapid sensing methods for IFN-γ have been developed including field effect transistor based biosensors (Bio-FETs) and Förster resonance energy transfer (FRET) aptamer beacons.6,14,15 These reported biosensing techniques display tremendous promise toward sensitive and rapid IFN-γ sensing with detection limits extending down into the nanomolar and even picomolar regimes. However, BioFETs that comprise micro/nano components require extensive cleanroom manufacturing steps (e.g., deposition of active source/drain regions, nano/micro wire gate development, gate oxide growth, metal contact and reference electrode deposition, and passivation layer formation) that substantially increase the cost of the biosensor and decrease its reproducibility (e.g., any crack/break in the passivation layer would interfere with the sensor signal during biological sensing).16 FRET-based aptamer beacon biosensors require labeling the target analyte with a fluorescent tag, a procedure that requires laboratory processing that is not conducive toward rapid in-field sensing. Likewise, FRET-efficiency depends on a variety of variables (e.g., nature of dyes, spacer length, or dye–dye interactions) that are difficult to characterize with in-field sensor equipment and highly variable with in-field biological samples.17,18 Electrochemical impedance-based biosensors circumvent these challenges by offering relatively facile fabrication procedures, label-free operation, and rapid (<35 min) in-field sensing.8,19–21 Furthermore, the use of an aptamer capture probe (ACP) as the biorecognition agent has shown promise in improving both the specificity and the sensitivity of more conventional antibody-based detection schemes.2,22,23 In fact, several aptasensors have been developed for the detection of IFN-γ.15,24–26 These biosensors show promise for rapid detection of IFN-γ; however, they require DNA hairpin structures and/or signaling probe labels that increase biosensor complexity and decrease the reliability of IFN-γ detection in actual serum samples.24,25 Some of these biosensors are also designed to detect IFN-γ at extremely low concentrations (down to picomolar ranges that are promising/necessary for tuberculosis detection/identification) while not demonstrating the capability to detect IFN-γ at higher concentration ranges that are necessary for potential John’s disease detection (considered positive when IFN-γ concentration is >1 ng/mL).26–29

Here we report the first interdigitated electrode (IDE) for IFN-γ detection with the use of immobilized RNA aptamers without the need for target analyte labeling. An Au IDE with 500 microbands (individual microband width = 5 μm) is used to detect IFN-γ via electrochemical impedance spectroscopy (EIS). It should be noted that such IDE-based biosensors experience advantageous mass transport and reaction kinetics effects (nonplanar or radial diffusion to each individual microband or IDE finger), higher current densities (electrical field lines are concentrated around tightly packed microbands), and favorable Faradaic-to-capacitive current ratios that lead to improved biosensor signal-to-noise ratios and hence higher sensitivity and lower detection limits for enhanced detection of antibody—antigen or aptamer—target binding events.30–34 A surface ternary monolayer consisting of 6-mercaptop-1-hexanolphosphate (MCH) /1,6-hexanediethiol (HDT) is also immobilized onto the Au IDE to impede nonspecific adsorption of nontarget, endogenous species and subsequently improve the biosensor selectivity. The immobilization of both the ACP and surface ternary monolayers onto the Au IDE is characterized by EIS in the presence of the redox couple [Fe(CN)₆]₃⁻/₄⁻ with EIS analysis and structurally characterized with the aid of atomic force spectroscopy (AFM). To better interpret the immobilization and sensing process, an equivalent circuit model is developed to explain in part the interfacial change between the electrolyte–electrode interface. This theoretical model validates the experimental data by elucidating the relationship between the biosensor signal output, viz., electrochemical impedance, and the double capacitance of both a bare (unfunctionalized) Au IDE and an Au IDE functionalized with the ACP/HDT-MCH surface ternary monolayer.

Methods

Chemicals. Bovine serum albumin (BSA), potassium chloride, Tris-EDTA buffer solution, 1,6-hexanediethiol (HDT), 6-mercapto-1-hexanolphosphate (MCH), buffered saline, postassium, Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), hydrogen peroxide solution (30% wt % in H₂O), sulfuric acid (95~98%), and hexaneoxyrate trihydrate were purchased from Sigma-Aldrich (USA). Fetal bovine serum (FBS) was obtained from GIBCO ThermoFisher Scientific while nucleic free water, potassium ferricyanide, and bovine IFN-γ recombinant protein (45 kDa) from ThermoFisher Scientific.

Au IDE preparation. An Au IDEs (Dropsens: G-IDEAU5) consisting of 500 fingers with both thickness and spacing of 5 μm and a finger length of 6760 μm were used as the biosensor electrode. The Au IDEs were cleaned with both Piranha solution (3:1 ratio of concentrated sulfuric acid vs hydrogen peroxide) and bath sonication (ethanol and then water for 30 min each respectively) and subsequently dried under an argon gas stream before ACP biofunctionalization.

Au IDE Biofunctionalization. The Au IDEs were functionalized with both an ACP probe and terminal monolayer (ACP/HDT-MCH) before electrochemical IFN-γ biosensing. First, an RNA ACP was purchased from Gene Link with a sequence of 5′-GGG AGG ACC ACG AUG CGG ACA CCG UUA AUC UGA GGC CCA GCC UCA GA-3′. This ACP was selected for its high binding affinity (Kᵦ = 18.7 ± 1.2 nM) to IFN-γ. The truncated sequence of the ACP eliminates nucleotide stretches that are not critical for direct interaction with the target, but can fold into the necessary structures to facilitate target binding. The terminal end was modified with a thiol SS-C6 group to enable thiol–gold binding to the Au IDE surface. The ACP probe was received in the lyophilized form and decapped with Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) by performing the following steps: 400 μL of 0.1 M TCEP was directly added into the lyophilized thiolated ACP and left at room temperature for 1 h with intermittent vortexing; next, 50 μL of 3 M sodium acetate at pH 5.2 and 1.5 mL of ethanol were added to the solution; finally, the resulting mixture was mixed at ~20 °C for 20 min to separate ACP from the solution; then, the solution was removed from the freezer and centrifuged at 12k rpm for 10 min to decant ethan and dry pellets in air. The decapped ACP was subsequently dissolved in 1 μM tris-HCl (TE) buffer. Au IDEs functionalized with ACP only were functionalized via drop coating with 20 μL of 500 nM ACP solution. Au IDEs functionalized with both ACP and HDT-MCH were created by first drop coating 20 μL of a solution containing 500 nM of ACP probe and 3 nM of HDT onto the Au IDE followed by dropcoating 20 μL of 1 mM MCH. Both distinctly functionalized Au IDEs were permitted to incubate for 30 min before rinsing thrice with nuclease free water and drying under a stream of inert argon gas to remove unbound/nonspecifically adsorbed IFN-γ. Finally, the functionalized Au IDEs were placed in a humidity chamber and stored at 4 °C in the dark overnight before electrochemical biosensing.

Electrochemical Detection of IFN-γ. Electrochemical impedance measurements were obtained from a CHI 6273E potentiostat (CHI instrument, USA), and a two-electrode setup was utilized (one side or one finger comb of the IDE as the working electrode and the other
side as the reference electrode). Functionalized Au IDEs were tested in a vial containing a 5 mM [Fe(CN)₆]³⁻/⁴⁻ redox probe and 0.1 M KCl salt within a 1× PBS (0.01 M) (pH 7.0) solution. Varying concentrations of IFN-γ (1, 2, 3, 4, 5, 10, 60, 100, and 200 ng/mL) were electrochemically detected by monitoring the impedance spectra/Nyquist plots using impedance spectroscopy ranging from 0.1 Hz to 100 kHz with an applied sine potential of 40 mV. Bovine IFN-γ recombinant protein diluted in 1× PBS with 1% BSA was used to perform the IFN-γ characterization tests. Before EIS measurements were obtained, the aptamer functionalized Au IDEs were incubated with the target antigen for 30 min before EIS sensing, which requires approximately 2 min. Therefore, the total aptasensor test time, including both incubation and electrochemical sensing time, requires less than 35 min.

**Electrochemical Interference Testing.** The Au IDEs biofunctionalized with ACP only and ACP/HDT-MCH were tested for sensitivity toward FBS and BSA. These interference tests were conducted by utilizing one of the following protocols: increasing the concentration of BSA (1, 100, and 1000 ng/mL) or increasing incubation times with pure FBS (5, 15, 30, and 60 min). EIS was subsequently utilized to evaluate the change of charge transfer resistance after said incubations.

# RESULTS AND DISCUSSION

**Au IDE Biofunctionalization Strategy.** The use of mixed self-assembled monolayers (SAMs) in conjunction with a thiol-derivatized DNA or aptamer capture probe and spacer thiols (e.g., MCH) functionalized on gold surfaces has been applied to improve both the hybridization efficiency and the reproducibility of the biosensors.38–41 These preliminary reports of mixed SAMs were composed of binary monolayers that suffered from surface defects (i.e., incomplete backfilling) that lead to unfavorable biosensor reproducibility. To circumvent these challenges research groups have added a third thiol component to help improve the filling density of the SAM and hence improve nonspecific adsorption of nontarget analyte to the Au surface.42,43 In this work, we utilize the HDT and MCH in conjunction with a RNA ACP to create a ternary surface monolayer that can be immobilized on the surface of the Au IDE to selectively detect IFN-γ in biological solutions (Figure 1). The Au IDEs were biofunctionalized with the ACP/HDT-MCH ternary surface monolayer via thiol-Au chemisorption following similarly published protocols and as explained in detail in the Methods section.42–45 Briefly, the ACP was subsequently co-immobilized with 1,6-hexanediol (HDT) to the Au IDE through thiol–Au interactions along with 6-mercaptop-1-hexanolphosphate (MCH) acting as a diluent. The dithiol HDT aligns horizontally with the Au surface instead of orthogonal, and subsequently more effectively backfills SAMs on Au surfaces compared to vertically aligned thiol groups, which cannot bridge over irregular/rough surfaces.37 Thus, this ternary surface monolayer has less nonspecificity from nontarget proteins than ternary monolayers produced with vertical thiol monolayers [e.g., cyclic dithiolethreitol (DTT)] or binary thiol monolayers.38–41

Furthermore, ACP was characterized on a smooth mica surface which permitted the structural characterization of the ACP (Supporting Information). The average thickness of the immobilized ACPs was measured to be 4.8 nm which correlates well with the height of the approximate 15 free base pairs (Bps) of the 3-loop RNA-based ACP (Figure S2a–f insets in Supporting Information; see ligand 2 F′P in ref 35, and note that the height of 15 RNA Bps is ~5.1 nm as each RNA Bp is ~0.34 nm). Also, the AFM characterization demonstrated that ACP immobilized onto the Au IDE along with HDT and MCH becomes saturated in terms of density when ACP from a stock concentration of 500 nm is used. Subsequently, this concentration of immobilized ternary monolayer (ACP/HDT-MCH) on the Au IDEs was used in the biofunctionalization protocols for IFN-γ detection. In order to assess the effects of the ternary surface monolayer on biosensor selectivity, Au IDEs were functionalized with the ACP only (Figure 1a) as well as with the ternary monolayer, ACP/HDT-MCH (Figure 1b).

**IFN-γ Detection Using Electrochemical Impedance Spectroscopy.** The developed aptasensors functionalized with (Figure 2a) and without (Figure 2b) HDT/MCH thiol backfillers were electrochemically characterized before IFN-γ sensing. Nyquist plots (imagine impedance −2″ versus real impedance Z′) displayed a semicircular pattern that increases in magnitude with the complexity of bioconjugation (ACP/HDT-MCH on AuIDE > ACP/HDT on AuIDE > bare AuIDE) (Figure 2a). The semicircle shape of the Nyquist plots corresponds to a direct electron transfer limited process.49 The modification of the working electrode surface, viz., the Au IDE, with an organic layer, viz., ACP, HDT, or MCH, tended to decrease the double layer capacitance and delayed the interfacial electron transfer rate as compared to a bare working electrode,51–53 as illustrated in Table 1. Furthermore, both the AuIDE biofunctionalized with ACP/HDT-MCH and ACP were tested with 10 ng/mL and 100 ng/mL of IFN-γ to demonstrate that the binding of IFN-γ to the aptasensor further decreased the double layer capacitance and delayed the interfacial electron transfer rate (Table 1). In other words, the ACP immobilized on the Au IDE was able to successfully bind to the target analyte (i.e., IFN-γ) and change the charge transfer resistance of the Au IDE in a measurable way with and without the presence of the thiolated backfillers (i.e., HDT and MCH) (Figure 2). Therefore, IFN-γ could still be measurably detected by the ACP functionalized electrode with the presence of HDT/MCH backfillers that tend to reduce noise from nonspecific adsorption and hence improves the signal-to-noise ratio of the sensor.
Next, the ACP (Figure 3a,c,e) and ACP/HDT-MCH (Figure 3b,d,f) functionalized AuIDEs were electrochemically characterized for IFN-\(\gamma\) sensing via EIS. Note that Figure 3b also shows the EIS response (albeit nearly negligible) for an unfunctionalized or bare electrode. The change of magnitude of the charge transfer resistance from the aptasensor without the thiol backfiller is much higher than with the backfiller, since there was nonspecific binding that occurred, which means the free-floating carrier protein from IFN-\(\gamma\) adhered to the exposed gold area. The collected data was then analyzed by plotting the percentage of change of charge transfer resistance (\(\Delta R\)) versus increased concentration of IFN-\(\gamma\) where \(\Delta R = R_c - R_o\) with \(R_c\) referring to the measured charge transfer resistance and \(R_o\) the charge transfer resistance of the electrode functionalized with ACP (Figure 3c–f). Linear regression analysis (see Figure S2 in Supporting Information) showed that both the ACP and ACP/HDT-MCH modified Au IDE electrodes displayed a linear sensing range across an IFN-\(\gamma\) concentration range of 22.22 pM to 0.22 nM (1–10 ng/mL) with a coefficient of determination Table 1. Generated Parameters from the Equivalent Circuit Model

<table>
<thead>
<tr>
<th>electrode functionalization(^a)</th>
<th>(R_c) (K(\Omega))</th>
<th>(n)</th>
<th>(K_0) ((\mu)m/s)</th>
<th>(Y) (1 (\times) 10(^{-7}))</th>
<th>(I) (nA)</th>
<th>(C_d) ((\mu)F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare</td>
<td>34.6</td>
<td>0.881</td>
<td>91.1</td>
<td>28.60</td>
<td>742.00</td>
<td>2.587</td>
</tr>
<tr>
<td>HDT + ACP</td>
<td>287</td>
<td>0.81</td>
<td>11</td>
<td>4.31</td>
<td>89.50</td>
<td>0.554</td>
</tr>
<tr>
<td>HDT + MCH + ACP</td>
<td>2030</td>
<td>0.865</td>
<td>1.55</td>
<td>0.86</td>
<td>12.70</td>
<td>0.300</td>
</tr>
<tr>
<td>10 ng/mL of IFN-(\gamma)</td>
<td>2690</td>
<td>0.972</td>
<td>1.17</td>
<td>2.82</td>
<td>9.53</td>
<td>0.274</td>
</tr>
<tr>
<td>20 ng/mL of IFN-(\gamma)</td>
<td>3380</td>
<td>0.787</td>
<td>0.932</td>
<td>0.99</td>
<td>7.60</td>
<td>0.032</td>
</tr>
<tr>
<td>30 ng/mL of IFN-(\gamma)</td>
<td>3560</td>
<td>0.974</td>
<td>0.884</td>
<td>2.73</td>
<td>7.20</td>
<td>0.031</td>
</tr>
<tr>
<td>40 ng/mL of IFN-(\gamma)</td>
<td>3930</td>
<td>0.83</td>
<td>0.801</td>
<td>0.83</td>
<td>6.53</td>
<td>0.028</td>
</tr>
<tr>
<td>50 ng/mL of IFN-(\gamma)</td>
<td>4120</td>
<td>0.976</td>
<td>0.765</td>
<td>2.63</td>
<td>6.24</td>
<td>0.026</td>
</tr>
<tr>
<td>100 ng/mL of IFN-(\gamma)</td>
<td>3410</td>
<td>0.977</td>
<td>0.924</td>
<td>2.40</td>
<td>7.53</td>
<td>0.026</td>
</tr>
<tr>
<td>150 ng/mL of IFN-(\gamma)</td>
<td>3560</td>
<td>0.974</td>
<td>0.884</td>
<td>2.73</td>
<td>7.20</td>
<td>0.031</td>
</tr>
</tbody>
</table>

\(^a\)HDT: 1,6-Hexanediol. MCH: 6-mercaptop-1-hexanolphosphate. ACP: Aptamer capture probe. IFN-\(\gamma\): Interferon-gamma.
A solution, the change of impedance saturates when the limit method that is commonly reported.\textsuperscript{47,48} Compared to the conventionally utilized instrument detection should be noted that the MDL method is more accurate from three standard deviations of seven distinct test trails. It should be noted that the MDL method is more accurate compared to the conventionally utilized instrument detection limit method that is commonly reported.\textsuperscript{37,49}

\textbf{Aptasensor Selectivity Experiments}. The ACP only and ACP/HDT-MCH functionalized Au IDEs were characterized for sensitivity to background interfering proteins including FBS and BSA (Figure 4). Figure 4a shows the electrochemical response of the aptasensors with increasing incubation times (5, 15, 30, and 60 min) of pure FBS (see Methods section). These selectivity experiments show that the change of $R_\text{ct}$ is negligible for the Au IDEs modified with the ternary monolayer ACP/HDT-MCH, while the Au IDE modified with ACP only exhibited increasing $R_\text{ct}$ in the presence of the target. Next, both the ACP and ACP/HDT-MCH functionalized Au IDEs were tested for their sensitivity toward solutions containing various concentrations of BSA (e.g., 1, 100, and 1000 ng/mL) (Figure 4b). Results of the BSA interference tests displayed similar results to those conducted with FBS in that the resulting $R_\text{ct}$ remained relatively constant (unchanged) with Au IDEs modified with ACP/HDT-MCH while those modified with ACP only did not. This higher specificity of the ACP/HDT-MCH functionalized Au IDE corroborates previous reports where DNA-based ternary monolayers showed similar higher specificity than electrodes without.\textsuperscript{37}

In order to further test the selectivity of the developed aptasensor, the ACP/HDT-MCH functionalized Au IDE was used to detect IFN-$\gamma$ in actual FBS solutions (Figure 4c,d). Figure 4c shows the change of impedance with increasing concentrations of IFN-$\gamma$ (1 ng/mL to 10 ng/mL) in FBS solution, the change of impedance saturates when the concentration reaches 6 ng/mL, while Figure 4d shows the linear sensing range is between 1 and 5 ng/mL [fitted line equation $y = 49.0x + 28.8$ ($R^2 = 0.95$)] with a theoretical detection limit of 0.52 ng/mL. Based on these results, this electrochemical platform could be used for detection of influenza induced respiratory infections (0.83 ng/mL),\textsuperscript{50} paratuberculosis (i.e., Johne's Disease in cattle, >1 ng/mL),\textsuperscript{27} tuberculosis pleural effusions (1.493 ng/mL),\textsuperscript{50} and multiple myeloma (1–10.7 ng/mL).\textsuperscript{51}

**Equivalent Circuit Model.** An equivalent circuit model was also created to help elucidate the physiochemical underpinnings of the obtained EIS experimental data (Figure 4). Such an equivalent circuit has been used in various forms to model the electrical/electrochemical behavior of interdigitated electrodes.\textsuperscript{52–54} Details regarding the setup of this model including key parameter definitions can be found in the Supporting Information; however, $R_\text{wire}$ and $R_\text{sol}$ parameters commonly found in such equivalent circuit models, were omitted because their values were negligible—the IDE-to-potentiostat connecting wire ($R_\text{wire}$) exhibited a negligible resistance (<1 $\Omega$) and the added solution electrolytes (i.e., KCl and PBS) lowered the $R_\text{sol}$ to a negligible level (~0 $\Omega$; note Nyquist plot starts at the origin (0,0) in Figures 2, 3, and 5). Also, the proposed equivalent circuit model portrays only one of the IDE finger comb electrodes (Figure 5a,b), as each of the IDE electrode finger combs are symmetrical and therefore modeling them both would be redundant. Furthermore, in this model, the $C_\beta$ was treated as a constant phase element (CPE) in lieu of an ideal capacitor.\textsuperscript{55} A CPE is a frequency dependent element that reciprocates between a resistor and a capacitor depending on the applied frequency and thus is a more accurate depiction of the behavior associated with an IDE.

The simulation output values ($R_\text{ct}$, $K_\alpha$, $n$, $Y$, $I$, $C_\beta$) are presented in Table 1 and the resultant model simulation Nyquist plot was fitted to experimental data (Figure 5c). Table

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Parameter & Value & Unit \\
\hline
$R_\text{ct}$ & 500 & $\Omega$ \\
$K_\alpha$ & 1 & \\
$n$ & 0.5 & \\
$Y$ & 1 & \\
$I$ & 1 & \\
$C_\beta$ & 100 & $\mu$F \\
\hline
\end{tabular}
\caption{Simulation parameters.}
\end{table}
calculate the charge transfer rate constant $K$ for monolayers such as MCH and HDT or nonspecific to the immobilization of blocking agents (e.g., thiolated rapid electron transfer rate on the bare electrode decreased due of nontarget proteins) on the simulated IDE surface. The $K$ is the temperature, and $n$ is the number of electrons. Finally, $C_\text{dl}$ was determined from $Y$ was equal to 0 when the CPE behaves like an ideal resistor (see eq 1). Also, note that $j$ is the imaginary number, $\omega$ is frequency, and $Q$ has the numerical value of the admittance $1/(\Omega)$ at $\omega = 1/\text{rad/s}$ in eq 1. The current exchange density $i_0$ needed to calculate the charge transfer rate constant $K_\text{ct}$ was evaluated from eq 2 where $R$ is the gas constant (8.3145 J/mol.K), $T$ is the temperature, and $n$ is the number of electrons. Subsequently, $K_\text{ct}$ was determined from eq 3 where $A$ is the area of the electrode, and $C$ is the concentration of the electrolyte. Finally, $C_\text{dl}$ is estimated from eq 4.

$$Y = \frac{1}{Q(j\omega)^n}$$

$$R_\text{ct} = \frac{RT}{nFi_0}$$

$$i_0 = nFAK_\text{ct}C$$

$$\omega = 2\pi f = \frac{1}{R_\text{ct}C_\text{dl}}$$

In summary, the proposed equivalent circuit model reveals the binding activity within the interface between the electrolyte and the electrode surface. For example, a bare or unfunctionalized Au IDE exhibits a $R_\text{ct}$ value of 34.6 KΩ, while Au IDEs functionalized with HDT/ACP or the ternary monolayer HDT/MCH/ACP exhibit much higher $R_\text{ct}$ values (287 KΩ and 2030 KΩ, respectively), which is in agreement with the fact that the rapid electron transfer rate on the bare electrode decreased due to the immobilization of blocking agents (e.g., thiolated monolayers such as MCH and HDT or nonspecific absorption of nontarget proteins) on the simulated IDE surface. The addition of IFN-γ also caused an increase of $R_\text{ct}$. For example, as the concentration of IFN-γ increased from 10 ng/mL to 50 ng/mL, the value of $R_\text{ct}$ increased from 2690 KΩ to 4120 KΩ. Since the $i_0$ (measured in units of nA) and $K_\text{ct}$ (measured in units of $\mu$m/s) are inversely proportional to the $R_\text{ct}$, the values of $i_0$ and $K_\text{ct}$ decrease when monolayers are added to the IFN-γ. Such chemical functionalization of the Au IDEs also leads to the decrease of double layer capacitance ($C_\text{dl}$) (Table 1). This decrease in double layer capacitance is most likely caused by the displacement of polar water molecules by less polar molecules near the electrode-electrolyte interface. It is also worth noting that the values of $n$ and the coefficient of CPE are all very close to 1 as reported in Table 1. Values of near-unity for $n$ highlight the fact that the double layer capacitance is acting more like an imperfect capacitor than a resistor during Au IDE electrochemical sensing—confirming that the model is depicting an electrical environment associated with actual conductive electrode/liquid electrolyte interfaces. Since the simulated results correlate well with the experimental results (Figure 5c), it is conceivable that such a model could be used to help facilitate the optimization of both the geometrical and biofunctionalization design of electrochemical IDE biosensors.

**CONCLUSIONS**

The developed ACP/HDT-MCH ternary surface monolayer on an Au IDE facilitates efficient hybridization with IFN-γ to reduce nonspecific adsorption of proteins found endogenously within FBS and BSA. This is the first report of developing an electrochemical IFN-γ biosensor with a ternary surface monolayer and the first electrochemical IFN-γ biosensor developed on an IDE array. This aptasensor is able to detect IFN-γ with a linear sensing region of 22.22 pM to 0.22 nM and lower detection limit of 20.22 pM (method detection limit technique) in buffer solution. In actual FBS solution, the aptasensor also detected IFN-γ with a linear sensing range from 22.22 pM to 0.11 nM with a detection limit of 11.56 pM. The ability of the biosensor to accurately detect IFN-γ in protein matrices without sample pretreatment or predetection labeling is well-suited for rapid in-field or on-penside biosensor detection strategies. The distinct ternary surface monolayer immobilized on multiple IDEs fabricated on a single silicon chip can be used for multiplexed biosensing for rapid on-site detection of diseases such as influenza induced respiratory infections, paratuberculosis (i.e., Johne’s Disease), tuberculosis pleural effusion, and multiple myeloma.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssensors.6b00581.

Atomic force microscopy (AFM) methods, aptamer chapter probe characterization via AFM, variability of electrochemical IFN-γ detection with the ACP and ACP/HDT-MCH functionalized AuIDEs, details regarding the equivalent circuit model (PDF)

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REFERENCES


