Optimization of Nanobelt Field Effect Transistor with a Capacitative Extended Gate for Use as a Biosensor

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In this study, various types of extended gate field effect transistor (EGFET) biosensors were compared and then a model based on potential coupling between a disposable extended gate (EG) capacitor and the gate-source/drain capacitor of a nanobelt field effect transistor (NBFET) was developed and optimized for biosensor applications. Several parameters, including the dielectric thicknesses and the EG area coverage, the ionic strength of buffer solution, and the charge density of specific binding molecules, were included in the model. The potential coupling efficiency between the potential induced by surface charge on the EG and the gate voltage of the FET was analyzed and verified through pH detection. Biotin–streptavidin/avidin sensing was demonstrated with the optimized EG and NBFET. In addition, real-time measurements of the detection of botulinum neural toxin (BoNT) type-A were also performed using the EG NBFET, which could detect an extremely low concentration (20 fM) of BoNT type-A. Because the nanoelectronic field effect transistor does not need to be sacrificed after detection, the optimized EG NBFET biosensor has great potential for use in bio/chemical and point-of-care applications.

Quantitative probing of surface charges at dielectric–electrolyte interfaces based on EGFETs has been examined. Moreover, various FETs—including Si-based FETs, GaN nanowire FETs, and In-Ga-Zn-O thin-film transistors—have been applied in EGFETs as biosensors for quantitative detection of various targets, including DNA, enzymes, hydrogen ions, and proteins. Nevertheless, no optimization analysis has been presented for EGFETs used as biosensors. In this study, various types of EGFETs (based on conductive EGs and capacitive EGs) were characterized initially for their suitability for use as biosensors. The performance of the capacitive EGFET biosensor was better in terms of holding the surface potential that resulted from specific binding. Therefore, employing capacitive-type EGFET biosensors, the potential coupling efficiency between the EG capacitor and the gate capacitor of the NBFET was optimized so that the surface potential, induced by the specific binding on the EG, could be sensed effectively by the NBFET. In addition, an NBFET with a near-ideal SS (ca. 62 mV/dec) was applied to serve as a transducer for optimal sensitivity, and an equivalent circuit model was developed to optimize the fabrication parameters for the EG. The optimized conditions for the EG allowed the detection of both streptavidin and avidin on biotin-modified EGs (surface modifications see supplemental information) using the EG NBFET. Finally, real-time detection of BoNT type-A was possible with detection achieved at concentrations as low as 20 fM. Moreover, the charge density of target molecules could also be estimated from the potentials measured using the proposed model.

**Experimental**

Comparative study of various types of EGFET biosensors.—Figures 1a and 1b depict schematic diagrams and simplified equivalent circuits of both conductive EGs and capacitive EGs with FETs in solution. Based on the equivalent circuit of a conductive EG, non-perfect electrodes result in unstable electrical characteristics in liquid environments, due to electrochemical reactions. Therefore, several kinds of insulator ion-sensitive films and membranes—including Gd2O3, TiO2, SnO2, and InGaZnO—have been examined as sensing dielectric layers for EGFETs.

With attractive features including high sensitivity, label-free detection, fast response times, and compatibility with complementary metal–oxide–semiconductor (CMOS) manufacturing, silicon nanowire (SiNW) field effect transistors (FETs) has been studied extensively as biosensors. Bio/chemical detection using SiNW FETs is based on the field effect through a very small gate capacitor after specific binding. SiNW FETs working at the subthreshold region have displayed the best sensitivity. The ideal subthreshold swing (SS) of a FET is approximately 60 mV/dec, meaning that a 60 mV potential change at the gate of the FET can induce a 10-fold change in current. There are, however, several obstacles hindering the real deployment of SiNW FETs as biosensors. First, despite the use of normalization techniques, device-to-device variations in transconductance can make it difficult to apply them in real quantitative analyses. Second, because of electrical variations [e.g., in threshold voltage (Vth)] among SiNW FETs, even for devices from the same batch, calibration is required prior to detection, increasing the time required per detection. Third, disposability of the SiNW FET after each detection event results in a very high cost per detection. As is the case for SiNW FET biosensors, the sensitivity of an extended gate (EG) FET biosensor is also influenced by the ionic strength (Debye-screening length) of the electrolyte solution and the transconductance of the FET. Unlike SiNW FET biosensors, however, EGFET biosensors are configured with a changeless FET and a disposable EG, providing more highly stable electrical characteristics and lower cost per detection. EGFET-based biosensors have several attractive features, including the capability of real-time, label-free detection and compatibility with modern integrated circuit (IC) technology. In particular, EGFET sensors are suitable for off-chip integration. Moreover, variations in surface modification, which is limited by two-dimensional random sequential adsorption, can be eased as a result of a large EG area, relative to that of a one-dimensional NW. The sensing electrode of an EGFET comprises a highly conductive material, which generates a signal from selective molecular recognition, and a FET structure, which transduces the molecular recognition event into an electrical signal. Typically, however, the use of highly conductive materials as sensing electrodes results in unstable electrical characteristics in liquid environments, due to electrochemical reactions. Therefore, several kinds of insulator ion-sensitive films and membranes—including Gd2O3, TiO2, SnO2, and InGaZnO—have been examined as sensing dielectric layers for EGFETs.
surface modifications can lead to a buildup of surface potential as a result of specific binding decay through contact between the solution and the conductive surface. The hold time of the buildup surface potential is strongly dependent on the quality of the modifications, especially the linkers, on the conductive surface. Figure 1c plots the decay of the surface potential with respect to the resistance of charge transfer, $R_{ct}$, in 0.01×phosphate-buffered saline (PBS). Figure S-1 displays the capacitance of electric double layer capacitor ($C_{dl}$) used in simulation for 0.01×PBS. A value of $R_{ct}$ of 1 GΩ was required to ensure effective detection and maintain a 90% surface potential within 3 min. The inset to Figure 1c presents the hold time (90%) of the surface potential plotted with respect to various values of $R_{ct}$. On the other hand, the capacitive EG, with the value of $R_{ct}$ usually greater than 10 GΩ, could maintain the buildup surface potential during the course of measurement. Figure 1d displays the values of $R_{ct}$ extracted from impedance analysis and curve-fitting using the equivalent circuit in Figure 1a for bare gold and subsequent modifications of gold surfaces with an 11-amino-1-undecanethiol (AUT) self-assembled monolayer and mouse IgG and anti-mouse IgG in 0.01×PBS. A value of $R_{ct}$ greater than 1 GΩ is highly unlikely through self-assembly on the conductive surface. Table S-1 (see Supplemental Information) lists the various types of EGFET biosensors that have been tested for proteins, DNA, glucose, and blood test species. EGFET biosensors with conductive EG usually exhibited detection limits at the nano- or micromolar level, far worse performance when compared with that of NW FET biosensors. Therefore, optimization of capacitive EG FETs as biosensors was conducted in this study in an attempt to equal the analytical performance of NW FET biosensors.

Simulations of sensing configurations.—The simulations were aimed at providing design parameters (e.g., thickness of dielectric layers; area coverage of EG on sensing chip) for EG fabrication so that a high potential coupling efficiency could be obtained between the EG and the gate of the NBFBET, as well as minimized surface potential interference from molecules binding outside the EG. Figures 2 displays a schematic representation of the EG and corresponding equivalent circuits molecules having total charge density of Q can be located either at the surface of the EG (Figures 2a and 2b) or at the surface outside the gate (Figures 2c and 2d). The surface charge density Q builds up the electric potential at the interface between the electrode surface and the electrolyte. Charges that resulted from specific binding of biomolecules were redistributed between the two parallel capacitors, $C_{dl}$ and $C_{Dry}$, as displayed in Figure 2. An aluminum wire connected the EG to the gate of the NBFBET so that the specific binding-induced potential could be sensed by the NBFBET. On the other hand, binding of molecules on the outside of the EG would also induce a gate potential on the NBFBET and cause an unwanted signal during real-time biomolecule detection. Therefore, the geometry and structure of the EG and the concentration of the electrolyte were optimized such that only the potential induced by specific binding on the EG would be coupled effectively to the gate of the NBFBET.

Fabrication of capacitive EG and NBFBET.—The EG NBFBET sensor comprised three main parts: a capacitive chip containing EGs for probe modification and capture of specific molecules, a microfluidic channel to deliver analytes onto the EGs (Figure S-2), and a corresponding NBFBET for each EG. The NBFBET transformed the change in surface potential after specific binding on the EG into a change in drain current. The sensing chip containing EGs was prepared on a Si wafer presenting a 2-μm-thick silicon dioxide layer. A 380-nm in situ–doped N⁺ poly-Si layer was deposited using low-pressure chemical vapor deposition (LPCVD) at 550°C. Next, the N⁺ poly-Si...
layer was defined into patterns having diameters of 0.5, 1, 1.5, 2, and 3 nm through optical lithography and reactive ion etching (RIE). A 10-nm-thick dry SiO2 layer was grown on the sensing chip using a horizontal furnace operated at 900 °C (see TEM image in Figure S-4). The NBFETs were prepared on a silicon-on-insulator (SOI) wafer having a 60-nm-thick top-Si. After cleaning, the wafer was implanted with phosphorus (3 × 10¹¹ cm⁻²) at 25 keV for use as the n+ region. The active region of the NBFET was defined using conventional optical lithography and etched through RIE. The channel length and width of the NBFET were 2 and 0.5 μm, respectively. To obtain high transconductance for the NBFET, the channel was thinned to approximately 7.2 nm using a thermal oxidation technique and a gate insulator (dry oxide) having a thickness of 6.6 nm was deposited through LPCVD (see TEM image in Figure S-4). The n+ regions [source/drain (S/D)] were prepared through self-aligned implantation with phosphorus at 3 × 10¹³ cm⁻² (25 keV) to decrease the contact resistance and series resistance at the S/D extension regions. A 150-nm-thick layer of heavily doped poly-silicon was deposited through LPCVD and defined through photolithography to form the gate of the NBFET. After forming the contact via, metallization and annealing in a forming gas at 400 °C was performed to obtain an ohmic contact. The on/off current ratio and the SS of the NBFET were approximately 10⁷ and 62 mV/dec, respectively (see Figure S-4).

Results and Discussion

Sensitivity of EG NBFET.—According to the schematic representation and the equivalent circuit of the floating substrate displayed in Figure 2a, the induced gate potential voltage (V_T) of the NBFET that resulted from the charge density (Q) of the specific binding on the EG can be described by Equation 1,

\[ V_T = \frac{C_{\text{Dry}}}{C_1 + C_{\text{Dry}}} \times V_{e,\text{typeA}} \]  \hspace{1cm} \text{(1)}

with \( V_{e,\text{typeA}} = \frac{Q}{C_{\text{Dry}} + C_{\text{Dry}}} \) and \( C_1 = \frac{C_{\text{Dry}} \times C_{\text{Dry}}}{C_{\text{Dry}} + C_{\text{Dry}} + (C_{\text{Dry}} \times C_{\text{Dry}})} + C_T \), where \( C_{\text{Dry}} \) is the capacitance between the dry layer and the sensing electrode; \( Q \) is the charge density of the specific binding biomolecule; \( C_{\text{Dry}} \) is the electric double-layer capacitance of the EG; \( C_{\text{Dry}} \) is the capacitance between the dry dielectric film thickness and the sensing chip; \( C_{\text{Dry}} \) and \( C_{\text{Dry}} \) are the electric double-layer capacitance and the bulk oxide capacitance outside the EG, respectively; and \( C_T \) is the gate capacitance of the NBFET device. We define the potential coupling efficiency as \( \eta = \frac{V_T}{V} \times 100 \% \), where \( V_T \) is the surface potential induced by the specific binding at the surface of the EG. We used two parameters to optimize the surface potential coupling efficiency (η): \( A_{\text{ratio}} \), the ratio of the areas of the EG and the total chip (A_Γ/A_Γ), and \( C_{\text{ratio}} \), the ratio of the capacitances of the gate capacitor of the NBFET and of the EG (\( C_T/C_{\text{Dry}} \)). Here, \( A_{\text{ratio}} \) also represents the surface coverage of the EG, while \( C_{\text{ratio}} \) represents the charge distribution between the EG and the NBFET device. In Equation 1, we assume that \( C_1 \) is approximately equal to \( \left( C_{\text{Dry}} \times C_{\text{Dry}} \right)/\left( C_{\text{Dry}} + C_{\text{Dry}} \right) + C_T \), because the value of \( C_{\text{Dry}} \) is usually 10 times larger than the value of \( C_{\text{Dry}} \) in an environment of high ionic strength (PBS > 1 mM). The bulk oxide thickness also affects the value of \( C_1 \); therefore, we also defined a ratio of the oxide thickness between the EG and bulk oxide dielectric film thickness \( x \) (D = x × d) to optimize the potential coupling efficiency, where \( d \) is the gate oxide thickness of the EG and \( \text{D} \) is the bulk oxide thickness of the sensing chip.

Figure 3 presents a plot of the potential coupling efficiency for a floating substrate configuration (Figure 2a) when charges were bound to the surface of the EG. Figures 3a and 3b display the potential coupling efficiency with respect to \( C_{\text{ratio}} \) and \( A_{\text{ratio}} \) for values of \( x \) of 1 and 1000, respectively. When \( x \) was equal to 1, as the value of \( C_{\text{ratio}} \) increased the value of \( \eta \) decreased, because of a voltage dividing effect between the two capacitors. A linear relationship existed between the charge density and the potential coupling efficiency.
As $A_{ratio}$ and $V_T$, because $A_{peak}$ is proportional to $C_{Dry}$. The black dashed line in Figure 3a reveals where the value of $\eta$ was greater than 90% on the left-hand side. The equation of this dashed line can be presented as $A_{ratio} = 1.5 \times C_{ratio} + 0.88$, for $0 < C_{ratio} \leq 0.08$. On the other hand, Figure 3b reveals that the region in which $\eta$ was greater than 90% increased when $x$ increased to 1000. Again, the equation of the dashed line can be described as $A_{ratio} = 9.5 \times C_{ratio} + 0.05$; $0 < C_{ratio} \leq 0.1$, as displayed in Figures 3b. A comparison of Figures 3a and 3b reveals that when the chip area ($A_{chip}$) remained constant, the value of $A_{ratio}$ could decrease 17-fold as the value of $x$ increased from 1 to 1000. Therefore, more EGs could be arranged on the same sensing chip by increasing the ratio of the oxide thicknesses of the bulk oxide and EG oxide. Such an arrangement would be helpful for multi-channel measurement using an EGFET. Figure 3c reveals that when the value of $C_{ratio}$ was 1, the change in $x$ had no significant effect on the value of $\eta$, with the maximum value of $\eta$ being approximately 50%. Figure 3d presents the impact of the value of $x$ in the type-A equivalent circuit when $C_{ratio}$ was equal to 0.01. We observed that the value of $\eta$ increased rapidly as $x$ increased from 0 to 4, due to the value of $C_{pro}$ being close to the value of $C_{Dry}$. Thus, when the value of $x$ was greater than 10, the values of both $C_{ext}$ and $C_{pro}$ decreased and a value of $\eta$ of least 80% could be obtained. Therefore, for the type-A configuration, a potential coupling efficiency of greater than 90%, resulting from specific binding on the EG, could be obtained effectively when $C_T$ was much less than $C_{Dry}$, and when $x$ was greater than 10.

The area outside of the EG could also capture the target biomolecules, and this additional signal could interfere with the surface potential measured by the NBFET. Therefore, we analyzed the type-B equivalent circuit, displayed in Figure 2c, using Equation 2

$$V_T = \frac{C_3}{C_4} \times V_{e, typeB}$$

with $V_{e, typeB} = \frac{Q}{C_{1} + C_{dis}}$.

$$C_3 = \frac{C_{ext} \times C_{pro} \times C_4}{(C_{ext} \times C_{pro}) + (C_{pro} \times C_4) + (C_{ext} \times C_4)}$$

and $C_4 = \frac{C_{dis} \times C_{Dry}}{C_{dis} + C_{Dry}} + C_T$.

Figures 4a and 4b display the potential coupling efficiency of the type-B equivalent circuit with respect to the values of $x$ and $A_{ratio}$ for values of $C_{ratio}$ of 0.01 and 1, respectively. The maximum of the potential coupling efficiency $\eta$ was approximately 48% when $C_{ratio}$ was equal to 0.01, when $A_{ratio}$ was close to 0.1, and $x$ was close to 1.1. The value of $\eta$ decreased dramatically however, when $C_{ratio}$ was equal to 1, due to the charge-induced potential being distributed only to $C_{ext}$ and $C_{pro}$. Moreover, the charges of the biomolecules located outside the EG had almost no effect on the value of $V_T$ when $x$ was greater than 10. Therefore, the value of $x$ was the most important design parameter for the EG in the type-B equivalent circuit. According to the simulations of both the type-A and type-B equivalent circuits, to obtain a high value of $\eta$ and minimize interference from charges binding outside the EG, the value of $C_{ratio}$ should be less than 0.1 and the value of $x$ should be greater than 10.

When the substrate of the EG was connected to the ground (Figure 2b), the potential coupling efficiency was similar to that of the type-A equivalent circuit. As a result of the grounding effect of the substrate of the EG, however, the impact of $x$ on the value of $\eta$ was not as dramatic as that in the type-A configuration. Because the value of $A_{ratio}$ was the same and $C_1$ (type A) was less than $C_T$ (type C), the value of $\eta$ could approach 100% only under the special condition that the sum of $C_{pro}$ and $C_T$ was much less than $C_{Dry}$. A higher value of $x$ was needed to give the same value of $\eta$ for the type-C configuration (see Figure S-5), relative to that in the type-A configuration. We also analyzed the type-D equivalent circuit (Figure 2d). As a result, when
all of the capacitors were connected to the ground, the charges of specific biomolecules binding outside the EG would not induce any change in the potential $V_T$. Under the same sensing conditions, type-A configuration provides the highest potential coupling efficiency.

Influence of ionic concentration and charge density.—The ionic concentration in the PBS solution has a significant impact on the sensitivity of both SiNW-FET sensors and NBFET sensors with EGs.\textsuperscript{15,34,35} In the analyte, the charged particles build up a surface potential through an electric double layer within the Debye length on the surface of the EG. This electric double layer also forms a capacitance $C_{dl}$ that is dependent on the ionic concentration. Any charge that emerged within the Debye length on the surface of the EG could induce a change in surface potential. The relationships among the value of $C_{dl}$, the Debye length, and the ionic concentration of the buffer solution are provided in Equations 3 and 4.

\[
C_{dl} = \varepsilon_0\varepsilon_r \times \frac{A_{ge}}{k^{-1}} \quad [3]
\]

where $\varepsilon_0$ is the permittivity in vacuum, $\varepsilon_r$ is the relative permittivity of the solution, and $k^{-1}$ is the solution’s Debye–Hückel length, which is strongly dependent on the ionic concentration:

\[
k^{-1} = \frac{\varepsilon_0\varepsilon_r k_B T}{2 N_A e^2 I} \quad [4]
\]

where $I$ is the ionic strength of the electrolyte (mol/m$^3$), $k_B$ is the Boltzmann constant, $T$ is the absolute temperature (in Kelvin), $N_A$ is the Avogadro number, and $e$ is the elementary charge. Although $C_{dl}$ can be estimated from Equations 3 and 4, the real value of $C_{dl}$ was extracted at various ionic strengths (from DI water to 1.51 M PBS) by measuring the two series capacitances $C_{dl}$, $C_{Dry}$ of the EG using an electrochemical impedance spectroscopy measurement system (BiosLogic, VSP-300). The capacitance of the series capacitor was saturated for ionic strengths greater than 10 mM, implying that the value of $C_{dl}$ was much greater than the value of $C_{Dry}$. The extracted capacitances $C_{dl}$ for the various ionic strengths (Figure S-1) were scaled with respect to the gate area and applied to all of the simulations.

Equations 1 and 2 and the measured values of $C_{dl}$ completely determine how the ionic concentration and charge density affect the potential coupling efficiency and the induced gate potential $V_T$ at the gate of the NBFET. Figure 4c displays the induced gate potentials of the NBFET for the type-A equivalent circuit with respect to the PBS concentration and for binding molecules of various charge densities on the EG for values of $A_{ratio}$, $C_{ratio}$, and $x$ of 0.5, 0.01, and 200, respectively. The ionic concentration affected the value of $C_{dl}$; in the case of a high-ionic-strength environment, the induced gate potential was very small at values of $A_{ratio}$ of 0.5, $d$ of 10 nm, $C_T/C_{Dry}$ of 0.01, and $x$ of 200. The value of $V_T$ increased $\sqrt{10}$ times, however, when the ionic strength decreased from 10 mM to 1 mM; accordingly, most studies of SiNW FET biosensors have used PBS buffer concentrations of less than 1 mM.\textsuperscript{12,36,37}

Surface charge densities at various values of pH.—We used pH measurements to verify the effect of the value of $C_{ratio}$. The electrical responses of the EG FET sensor were measured at various values of pH using an Agilent 4156C apparatus. The areas (capacitance) of the tested circular EGs were 0.196 (0.69 nF), 0.785 (2.37 nF), 1.77 (5.4 nF), 3.14 (9.28 nF), and 7.06 (20.7 nF) mm$^2$, respectively. The chip area outside the EG, $A_{ext}$, was 37 mm$^2$, defined by a polydimethylsiloxane (PDMS) well having a diameter of 8 mm. An ultra-low level commercial MOSFET (Philips-PH2520U) having a large output capacitance ($C_T$) of 12 nF was adopted for this experiment. The bias of S/D voltage was fixed at 10 mV. The potential bias at the reference electrode (Ag/AgCl electrode) was set to give a drain current of 1 nA at pH 10. Figure 5a presents the real-time drain currents ($I_D$) of the EG FETs when flowing PBS buffers at values of pH from 10 to 4 over the silicon dioxide surfaces of the EGs. According to the Nernst limit, the
theoretical sensitivity to the pH is approximately 59 mV/pH at room temperature. Protonation and deprotonation of surface silanol groups causes a change in the surface potential, SiOH₂⁺ ⇔ SiOH + H⁺ and SiOH ⇔ SiO⁻ + H⁺. The best conditions for sensing were for the EG having the largest value of Cratio (for Aṇo = 7.06 mm² and CDry = 20.7 nF), which provided the largest change in the value of ID for each change in pH. This measurement was consistent with the simulation data: an increase in the value of Aratio results in a decrease in the value of Cratio and an increase in the value of VT. The corresponding changes in charge density could also be obtained using Equation 1, as displayed in Figure 5c. Assuming the point of zero charge of the SiO₂ surface in 0.01 × PBS occurred near pH 4,¹⁹ when the negative surface charge density increased from pH 4 to 10, the surface charge density reached ~0.5 C/m² at pH 10.

Figure 5b presents the real-time drain currents (ID) of the EG FETs after flowing PBS buffers at values of pH from 10 to 4 over the APTES-modified EGs. The NH₂ groups on the surface allowed detection of the pH₁ –NH₂⁺ ⇔ –NH₂ + H⁺. Compared with the SiO₂ surface, the pH response was smaller, but the linearity in response to the pH level was enhanced. The negative surface charge density increased from 0 to ~0.15 C/m² when the pH increased from 4 to 10, as displayed in Figure 5d. The smaller change in surface charge density might have resulted from the reaction of one APTES molecule with three silanol groups on the SiO₂ surface; that is, fewer active functional groups were available for reaction with the various pH buffers. Compared with the silanol surface, the APTES-modified surface exhibited a change in charge density of only approximately 30%.

**Biotinylation and BoNT type-A detection.**—To examine the performance of the optimized EG NBFETs, the biotin–streptavidin/avidin interaction was measured using the best experimental setup: an EG having values of Aṇo of 7.06 mm², CDry of 20 nF, and x of 200, and an extremely sensitive NBFET (SS = 62 mV/dec; CT = 3 pF). Biotin binds to both streptavidin and avidin rapidly with high affinities, high specificities, and low dissociation constants (kd).³⁸ Moreover, streptavidin and avidin have quite similar characteristics, apart from their isoelectric points (PI; streptavidin, 5.0; avidin, 10).³⁹,⁴⁰ Figure 6a displays the sensing responses of the EG NBFETs toward streptavidin and avidin using the biotin-modified EG in PBS at pH 7.4. The fluid delivery system featured a PDMS microfluidic channel, a flow rate of 50 μL/min, and poly(methyl methacrylate) (PMMA) clamps. Analytes were delivered by a syringe pump through Teflon tubes (Figure S-2(b)). The sensing measurement was performed through sequential injection of streptavidin at concentrations from 0.42 pM to 42 nM over the biotin-modified EG. The measured current ID was transformed into the surface potential. The binding of streptavidin on the electrode surface induced a negative potential, due to its PI of 5.0. Similarly, avidin was injected over the other EG at concentrations ranging from...
Figure 6. (a) Real-time measurements of various concentrations of streptavidin and avidin using an EGFET. (b) Real-time sensing of BoNT type-A using an EGNBFET featuring EGs modified with type-A BoNT Ab. (c) Numerical simulation of gate potentials for EGNBFETs, plotted with respect to charge density and PBS concentration; the red star indicates the surface potential measured from real-time sensing of 20 pM BoNT type-A. (d) Surface potential changes of BoNT and interference protein HSA using BoNTs antibody modified EG. All measurements were performed at constant ionic strength (1.5 mM PBS solutions).

0.35 pM to 35 nM. The response was an electric surface potential opposite to that obtained for streptavidin binding. The measured changes in surface potential were −12 and +7.6 mV at a streptavidin concentration of 42 nM and an avidin concentration of 35 nM, respectively.

To demonstrate the applicability of the proposed EGNBFETs as sensitive biosensors, we investigated their ability to detect BoNT type-A. Figure S-6 displays the modification of the BoNT type-A antibody on the EG. The real-time measurement was conducted in three stages (Figure 6b): injection of 0.01 × PBS (pH 7.4, 1.5 mM) for 3 min at a flow rate of 50 μL/min, to establish a baseline; injection of the BoNT type-A at concentrations from 0.2 fM to 20 pM; and washing with 0.01 × PBS to dissociate any weakly binding BoNT type-A. As a result, the change in surface potential between stages 1 and 3 would correspond to the binding of BoNT type-A on the surface of the EG. Figure 6c displays a simulation of the induced values of $V_T$ at various PBS concentrations and different binding charge densities. The red star in Figure 6c denotes the change in surface potential (ca. 6 mV) for the detection of 20 pM BoNT type-A. The estimated charge density was approximately 1.87 mC/m², implying a binding charge of 13.2 nC on the EG. Figure 6d displays the Surface potential changes of BoNT and interference protein human serum albumin (HSA) using BoNTs antibody modified EG showing that the interference from HSA was minimal. As a result, HSA at various concentrations (0.2, 2, and 20 nM) was examined as an interference protein for the EGNBFET with the BoNT type-A antibody–modified EG. Minimal interference from HSA was evident, revealing good selectivity between BoNT type-A and the antibody (see Figure S-7). Moreover, compared with other BoNT detection techniques, the proposed method provides rapid detection with a detection limit as low as 20 fM. With the optimized design, this EGNBFET exhibited analytical capabilities equal to that of SiNW FET biosensors.

Conclusions

A comparative study of conductive and capacitive EGFETs as biosensors has led to the development of a simulation model based on potential coupling between an EG capacitor and the gate–source/drain capacitor of an NBFET that could be used to optimize biosensors. Optimization of the proposed model provided parameters for EG fabrication, the ratio between EG capacitance and the gate capacitance of the NBFET ($C_{ratio}$), and the ionic strength of the working buffer solution to ensure high potential coupling efficiency. The charge density of specific binding biomolecules could be estimated from the potential induced at the gate of the NBFET. To obtain values of $\eta$...
of greater than 90% and minimize interference from charges located outside the EG electrode, it was found that the value of $C_{ratio}$ should be less than 0.1, the oxide thickness ratio between the gate and bulk oxide dielectric film of the EG should be at least 10, and the ionic concentration should be less than 1.5 nM. The model was verified through sensing of biotin–streptavidin/avidin conjugation using the EG NBFET. Furthermore, the optimized EG NBFET was used to obtain real-time measurements of the detection of BoNT type-A. The EG NBFET sensor could detect an extremely low concentration (20 fM) of BoNT type-A within 20 min. Because the nanoelectronic device would not need to be sacrificed after detection, the configuration of this EG NBFET biosensor has great potential for use in biochemical and point-of-care applications.

Acknowledgments

We thank the Ministry of Science and Technology of Taiwan (MOST 104-2221-E-009-117-MY2).

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