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Surface Charge Control and Drift Suppression of Graphene FET for Ultra-Sensitive Detection of Viruses

BY

KAORI YAMAMOTO

MARCH 2026

Surface Charge Control and Drift Suppression of Graphene FET for Ultra-Sensitive Detection of Viruses

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BY

KAORI YAMAMOTO

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Abstract

Graphene have two great features such as a high mobility of $\sim 200,000 \text{ cm}^2/\text{Vs}$ compared to the conventional semiconductors such as silicon semiconductors with that of $1,350 \text{ cm}^2/\text{Vs}$, and 2-dimensional electron gas (2DEG) is on its surface. Also, graphene is stable even in the solution, and is easy to be modified by various receptors. As carbon atoms of graphene are exposed to the surface, it is easy to immobilize various receptors via non-covalent bonding such as π - π interactions. These distinctive properties render graphene a highly sensitive sensing platform.

By using these advantages, we have been developing Graphene Field-Effect Transistors (GFETs) biosensor. In my study, SARS-CoV-2 and avian H9N2 influenza virus were successfully detected with high-speed and high-sensitivity. Various challenges were overcome to achieve high-sensitivity detection.

Firstly, we found that the control of the surface charge density of graphene is important to get the highly sensitive detection. The sensitivity reaches its maximum when the surface charge density is close to zero based on the theory from the equation combining the formula of Poisson equation and Boltzmann diffusion. To realize this condition, we propose the new method, i.e., the polymer carrying positive charge, PLL, was added onto GFET to make the surface charge density of graphene approach to zero. Using this method, we have succeeded in enhancing 10 times higher sensitivity for the detection of SARS-CoV-2 compared to the same detection without PLL.

Secondly, it was found that the virus can be detected in acetate buffer without cationic polymer. Surprisingly, the sensitivity of GFET with cationic polymer in acetate buffer was decreased. These results obey the theory.

Thirdly, we found that the V_{DP} drift in the bio sensing is attributed to the drift of the leakage current between the reference electrode and source/drain metal electrodes. By suppressing this gate leak current using the SiO_2 passivation layer, we have succeeded in reducing the drift effect in the virus measurement, and consequently, high-speed and high-sensitivity virus detection was successfully achieved.

In conclusion, we developed highly sensitive GFET biosensor using these research and discovery.

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1 General introduction

1.1 Research background

Rapid and simple virus detection methods are highly required. Though highly sensitive PCR tests were widely used during COVID-19 pandemic¹⁻⁴ they had the disadvantages of requiring several hours to obtain results. Meanwhile, results of antigen test using immunochromatography are obtained rapidly but false-negative results sometimes occur when viral concentration in a specimen is insufficient⁵⁻⁷. We have tried to develop a biosensor with higher sensitivity and faster measurement. Although graphene is an innovative material because of its electrical and physicochemical properties, it has not yet reached full-scale commercial applications because of mass production, cost reduction, and quality stabilization. To date, application development in electronic devices, energy storage systems, composite materials, and medical fields has been developing.

1.2 Graphene

Graphene is 2-dimensional thin layer and its thickness is approximately 0.34 nm. This thickness corresponds to atomic one layer. The carbon atoms are arranged in a honeycomb-like lattice structure of sp^2 -bonded carbon atoms as shown in Fig. 1.1. The C-C bond length is approximately 0.142 nm. A. Geim and K. Novoselov successfully isolated a monolayer graphene sheet from bulk graphite for the first time in 2004⁸ and received Nobel Prize in 2010. They develop the method to obtain graphene monolayer using scotch tape. This method has called mechanical peeling method. But this graphene size is on the order of several tens of micrometers. Currently, graphene have grown by chemical vapor deposition (CVD). In CVD method, graphene is grown on Cu foil by reacting gas such as methane etc. at approximately 1000 °C. This method enables graphene to be glowed in large area and high quality. Graphene is well known to have various features and shown below. Graphene have a high mobility of $\sim 200,000 \text{ cm}^2/\text{Vs}$ compared to conventional semiconductors such as silicon semiconductors with that of $1,350 \text{ cm}^2/\text{Vs}$ ^{9,10}, and 2-dimensional electron gas (2DEG) is on its surface, etc. Also, graphene is stable even in the solution, and is easy to be modified by various receptors¹¹⁻¹³. Carbon atoms of graphene are exposed to the surface, it is easy to immobilize various receptors via non-covalent bonding such as π - π interactions^{14,15}. These distinctive properties render graphene a highly sensitive sensing platform.

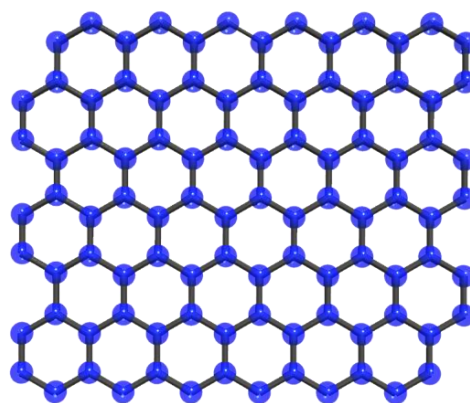


Fig.1.1. Illustration of single layer graphene.

1.3 Structure and operation principle of graphene field-effect transistors

1.3.1 Graphene field-effect transistor

By using these advantages written in Section 1.2, we have been developing Graphene Field-Effect Transistors (GFETs) biosensor to detect the various viruses, ions¹⁶⁻¹⁸, gases¹⁹⁻²¹, proteins²²⁻²⁴, nucleic acids, and exosomes²⁵ etc. GFET is a device which uses a monolayer graphene as the channel of the MOS (Metal Oxide Semiconductor) transistors. Fig. 1.2(a) shows a schematic illustration of GFET. SiO₂ thin oxide layer is 290 nm thermal oxide film as insulating layer. Source/drain electrodes using Ti (90 nm)/Au (10 nm) was formed on SiO₂ layer. Graphene is put on the channel between the source/drain electrodes. The optical image will be shown later. Moreover, functionalizing GFET is straightforward and safe, since compounds containing π electrons dissolve in solution and bind to graphene via π - π interactions. Thus, GFET sensors have versatile applications. Moreover, the present GFET shows rapid detection by measuring only the binding time between virus and the receptor, plus small time of the transfer characteristics before and after binding.

Fig. 1.2(b) represents the typical transfer characteristic of GFET. The horizontal axis is the gate voltage V_{GS} and the vertical axis is the drain current I_{SD} . Dirac point (DP) is defined as the V_{GS} at the lowest I_{SD} . The carrier density is 0 at DP. In the left of the DP, holes are induced in graphene that works as carriers. On the other hand, in the right of the DP, electrons are induced in graphene that works as carriers, too. Therefore, the shape of transfer characteristics of GFET is ambipolar characteristic. Also, GFET is a zero-bandgap semiconductor and does not have OFF state.

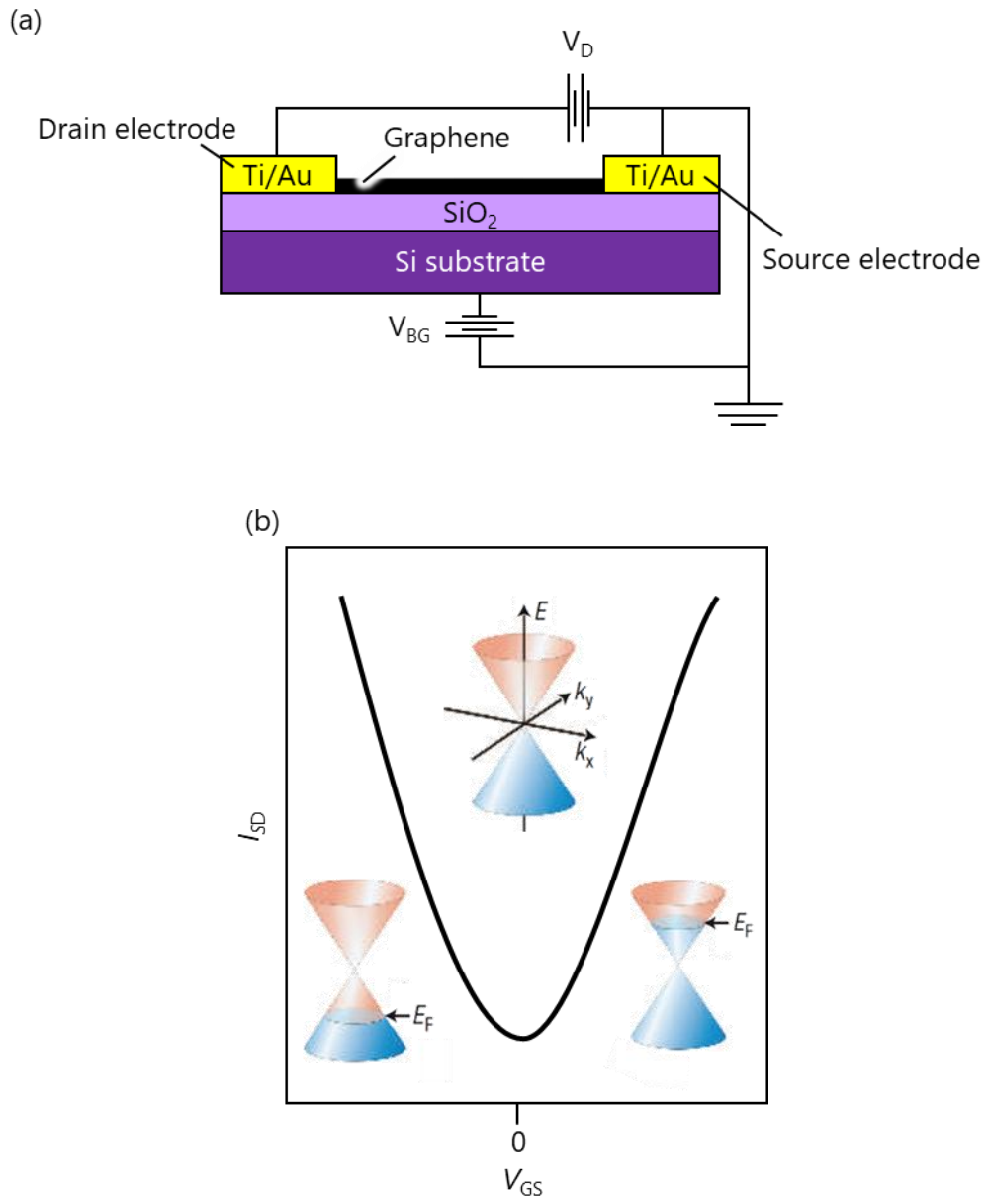


Fig. 1.2. (a) Schematic illustration of graphene field-effect transistor (GFET). (b) Typical transfer characteristics of GFET.

1.3.2 Solution-gate FET sensor

To date, various materials have been employed for solution-gate FETs. Hu et al. employed a SiNW-FET for pH measurement²⁶, while Chakraborty et al. detected antigens using a ZnO nanorod FET²⁷. Compared with these devices, GFETs can detect diverse targets with high sensitivity, owing to graphene's high electron mobility and the exposure of its 2DEG at the surface to the electrolyte. Moreover, many biosensing methods using FET are introducing electrolyte with virus to FET containing electrolyte without virus or with virus at a lower concentration in advance. In this environment, many viruses are suspended in the electrolyte between a reference electrode and FET, and some viruses are physically adsorbed on the GFET. Therefore, accurate measurement was impossible, so far. However, in the present research, viruses only binding to antibody (Ab) can be detected by our sensors because viruses in electrolyte are removed using a microchannel and a micro pump system.

1.3.3 Solution-gate GFET sensor

A schematic image of solution-gate GFET is shown in Fig. 1.3(a). Microchannel system or silicone rubber pool are set on GFET, and electrolyte is introduced to it. An Ag/AgCl reference electrode is employed to apply the V_{GS} . This structure is called solution-gate GFET. If the voltage was applied to reference electrode, ions in electrolyte move as follows. If positive voltage was applied to reference electrode, negative charges in electrolyte are accumulated near the electrode. Conversely, positive charges in electrolyte are accumulated near the graphene surface. Fig. 1.3(b) represents the interface between graphene and electrolyte. The positive charges in electrolyte and the negative charges in graphene are in contact and equal amounts. This is called electric double layer.

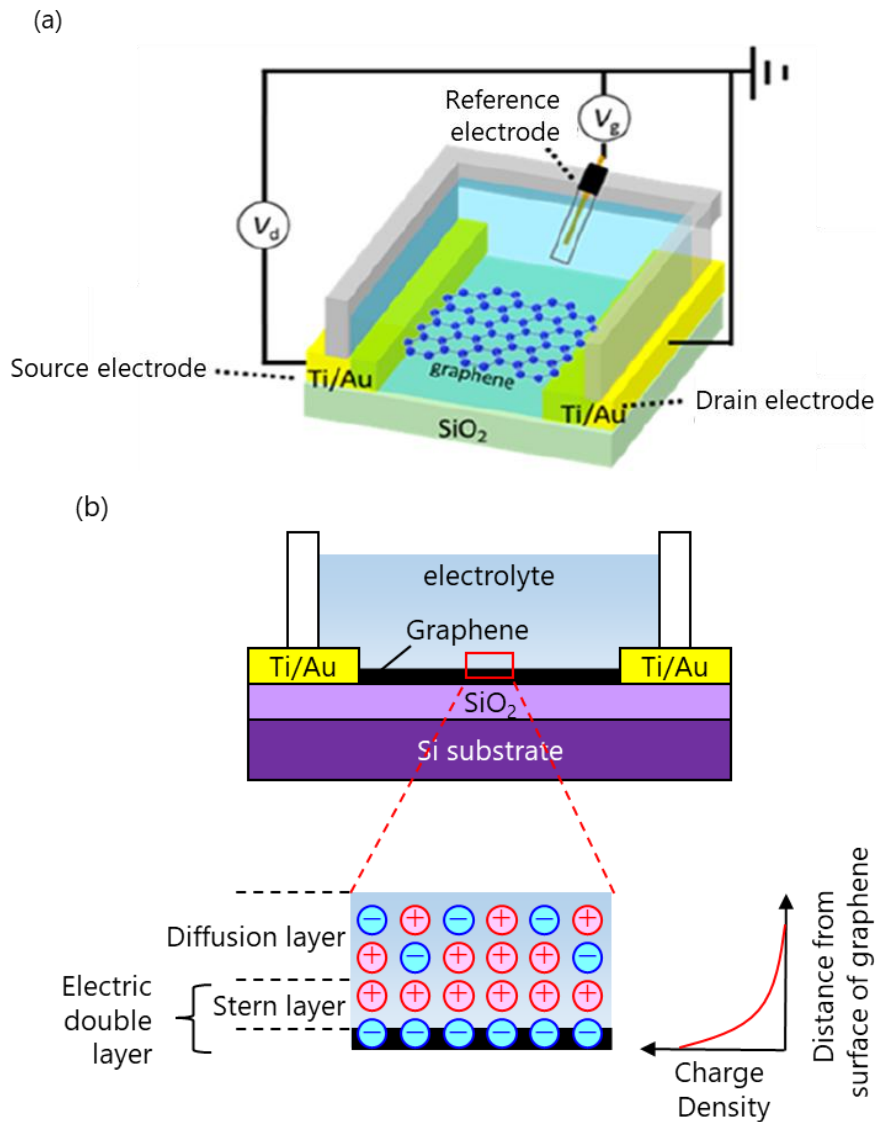


Fig. 1.3. (a) Schematic image of solution-gate graphene field-effect transistor. (b) Interface between graphene and electrolyte.

1.4 Research purposes

In this study, we will describe GFET biosensor, and research purposes are as follows:

- (1) Enhance the sensitivity of GFET biosensor by controlling the surface charge density
- (2) Suppress the drift characteristic for the stable measurement

- (1) Enhance the sensitivity of GFET biosensor by controlling the surface charge density

Until now, we had developed GFET biosensor using the mechanical peeling graphene as shown in Fig. 1.4. However, this GFET had two issues; (i) the fabrication process is highly time-consuming, and (ii) one GFET can be used for only one virus measurement. To resolve these issues, CVD graphene was employed and GFETs array was formed on a silicone substrate. Using CVD graphene, Large-area graphene transfer became possible. Forming GFET array, probe FETs and reference FETs can be fabricated on same substrate. These methods enabled calculating 1) the means and the standard deviations, 2) cancelling out the effect of the physical adsorbed virus, and 3) reduction of the drift effect. When SARS-CoV-2 Ab was modified on GFET as probe FET and influenza Ab was modified as reference FET, then SARS-CoV-2 virus was introduced in all GFET, only the transfer characteristics of GFET with SARS-CoV-2 Ab shift because virus bind with its Ab only specifically interact under ideal conditions. When the sensitivity of GFET is low, sometimes the virus charge could not be detected. To enhance the sensitivity, poly-L-lysine (PLL) was employed based on Poisson-Boltzmann equation. ζ potential of graphene is negative, and the sensitivity becomes highest when the surface charge density of graphene is 0. Thus, PLL with positive charge over a wide pH range was applied to GFET to make the surface charge density of GFET near zero to get the highest sensitivity.

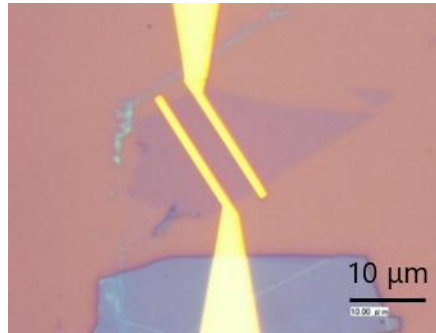


Fig. 1.4. Optical image of graphene field-effect transistor using mechanical peeling graphene.

- (2) Suppress the drift characteristic for the stable measurement

As described above, one of the issues of GFET biosensor is the drift in the transfer characteristics. To solve this problem, the passivation layer was fabricated to insulate the gate leakage current from reference electrode to the source/drain electrode of GFET. As a result, the drift of the transfer characteristic could be suppressed.

1.5 Outline

In Section 1, the general property of graphene and GFET was described. Graphene and GFET structures were also described. Moreover, the environment surrounding FET biosensor in recent years and position of GFET biosensor as a research background will be shown.

In Section 2, the properties of the graphene material were described.

In Section 3, the basics of the virus detection was described. The fabrication process of GFET and Ab modification methods were described too. In this section, 1×10^8 FFU/mL SARS-CoV-2 virus was detected.

In Section 4, the relationship between the surface charge density of graphene and the electrode potential in the solution was calculated using Poisson equation and Boltzmann distribution, and the sensitivity of the graphene FET biosensor was derived. This equation leads us to success in the virus detection with higher sensitivity.

In Section 5, based on the equation obtained in Section 4, polymer with cation was introduced into the GFET biosensor, and 1×10^7 FFU/mL SARS-CoV-2 virus was detected with ten times higher sensitivity. Thus, the sensitivity of GFET biosensor could be enhanced by polymer with cation. Moreover, acetate buffer was employed instead of Phosphate-buffered saline (PBS) as measurement buffer. The purpose is to enhance the charge of virus by the lower pH. As a result, virus was detected in acetate buffer using GFET without polymer because the surface charge density of graphene is near to 0 due to positive charge of Ab in the acetate buffer at pH 4. These experimental results were considered well with the equation obtained in Section 4.

In Section 6, the new method to suppress drift characteristics in the GFET biosensor was described. In Section 6, by insulating the metal source/drain electrodes with the passivation layer, the drift characteristics were suppressed and obtained measurement results more quickly and high sensitivity.

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2 Biosensing application using functionalized GFET

2.1 Introduction of functionalized GFET biosensor

There are many studies of GFET biosensors which tries to detect SARS-CoV-2 spike proteins. For example, E. Piccinini et al. detected spike protein using Ab-modified GFETs, with a limit of detection (LOD) of 6.7 nM¹. A. Silvestri et al. also detected spike protein using angiotensin-converting enzyme 2 (ACE2) modified GFETs, with a LOD of 2.4 aM². While many studies have employed spike proteins as the sample, only a few have demonstrated the detection of whole viruses³. Furthermore, in most of the viral measurement studies so far reported include some problems, such as the electrolyte during the viral measurement includes many viruses, and some viruses are physically adsorbed on the element⁴⁻⁶. However, viruses only binding to Ab can be detected by our sensor because viruses in electrolyte are removed using a microchannel and a micro pump. Furthermore, in most of the studies, a single GFET sensor was used for virus detection, despite the fact that the electric characteristics of GFETs vary significantly due to the process variation⁷, thereby rendering results based on single GFET measurements potentially controversial. Moreover, baseline drift is a well-known drawback of GFET biosensors because the drift makes it difficult to distinguish between signal and noise^{8,9}. Nonetheless, the issue is often dismissed and not discussed.

2.2 Purpose

In the present study, we aim to develop a more accurate measurement system with higher sensitivity for the biosensing using GFET. Firstly, CVD graphene was employed instead of the mechanical peeling graphene and 32 GFET array were fabricated on a single silicone substrate as described in Section 1.3. By using the large size of CVD graphene, GFET array could be formed, and probe FETs and reference FETs are fabricated on a substrate. These methods enabled us to calculate 1) the mean values and the standard deviations of the measurement results, 2) cancelling out the effect of the physical adsorbed virus on graphene, 3) reduction of the drift effect. These methods enable the accurate biosensing compared to studies by others. Secondly, microchannel system made by PDMS was employed onto the GFET array. As a result, reproducibility of the biosensing measurement was improved because the operation becomes synthesized for any operator. Consequently, microchannel system enables virus detection at home in the future.

2.3 Procedure of GFET fabrication

2.3.1 Design configuration of electrode

Source and drain electrodes were formed by depositing 10 nm/90 nm of Ti/Au on a SiO₂/Si substrate. An optical image of the substrate is shown in Fig. 2.1. After depositing gold on titanium over the entire surface, G-line resist purchased from Tokyo ohka kogyo Co., Ltd. was coated. After exposure via quartz photomask, a part of photoresist was removed by developer (TMAH 2.38%). Ti/Au was removed by nitric acid (AURUM 306) and all resist was removed.

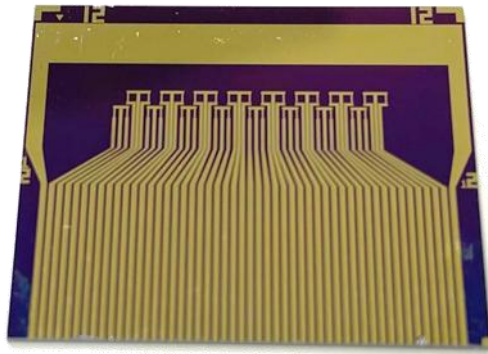


Fig. 2.1. Optical image of silicone substrate with Ti/Au metal electrodes.

2.3.2 Transferring of graphene

The graphene film was transferred using poly methyl methacrylate (PMMA) onto the prepared SiO₂/Si substrate. Graphene with Cu foil and PMMA A7 were purchased from Institute for 2D materials LLC. and Nippon kayaku Co., Ltd. After the PMMA coat on graphene with Cu foil at 6000 rpm and 60 second using spin coater as shown in Fig. 2.2, Cu was dissolved in ammonium persulfate. Subsequently, PMMA on graphene was transferred on prepared silicone substrate after PMMA on graphene rinsed with pure water.



Fig.2.2. Spin coater.

2.3.3 Plasma etching and removal of PMMA

After drying graphene with PMMA on silicone substrate under ambient conditions, it patterned via oxygen plasma etching as shown in Fig. 2.3, resulting in the integration of 32 GFETs on the substrate. The PMMA film was dissolved in ethanol.



Fig. 2.3. Reactive ion etching (RIE).

2.3.4 Results of fabricated GFET

GFETs were arranged in an array of two rows and 16 columns, and numbered from #0 to #31, as depicted in Fig. 2.4. Optical images of a typical GFET will be shown in each section.

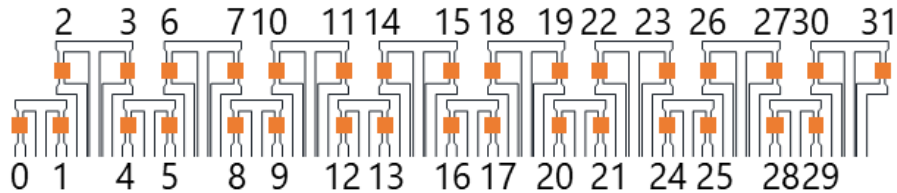


Fig. 2.4. Schematic of the graphene field-effect transistor (GFET) arrangement on the electrode pattern. GFETs were numbered from #0 to #31.

2.4 Measurement conditions and measurement results

2.4.1 Measurement conditions

All measurements were performed using custom-built equipment shown in Fig. 2.5 and the source-drain bias V_{SD} of 100 mV was applied. Top gate voltage from Ag/AgCl reference electrode and electrolyte will be described in each section. The gate voltage was applied to GFET via electrolyte as described in Section 1.3.3.



Fig 2.5. Optical image of custom-built equipment for measurement of transfer characteristics

2.4.2 Results of transfer characteristics

Typical results of transfer characteristics were shown in Fig. 2.6. Each line represents one of FETs #0 to #31 in Fig. 2.4, arranged in rainbow order from red to blue. 150 mM PBS was used as electrolyte. V_{GS} was swiped from -300 to 300 mV. The DP is not at 0V because of the effect such as the PMMA residue and O_2 molecule on graphene, etc. Zero I_{SD} indicates that the graphene layer may have peeled off. The GFET with low g_m (green line) observed in some FETs, and is attributed to the graphene tearing off at the gold–substrate step.

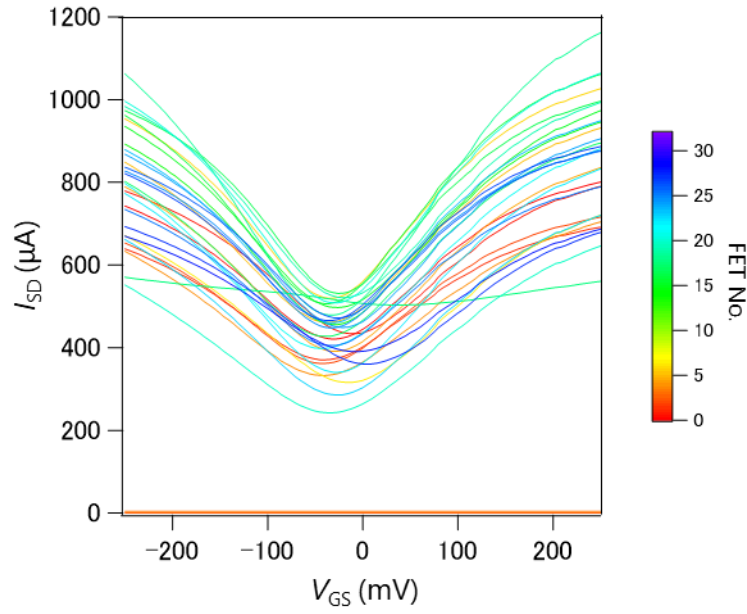


Fig. 2.6. Typical results of transfer characteristics. Each line represents one of graphene field-effect transistors arranged in rainbow order from red to blue.

2.4.3 Results of Raman spectra

Raman spectrum of graphene is important for GFET measurement because it evaluates the defects present in the graphene. The defect of the graphene may lead low sensitivity of GFET. G band originated from the in-plane vibrations of carbon atoms and appears near 1580 cm^{-1} . 2D (G') band arises from a second-order two-phonon scattering process and appears near 2700 cm^{-1} . D band is known as the band, arising from structural disorder and defects. If the band presence in the range of $1270\text{--}1450\text{ cm}^{-1}$, it indicates that defects or edges in the graphene sample. Raman spectrum of graphene after GFET formation was measured as shown in Fig. 2.7. As can be seen, G and 2D bands are observed. Conversely, D band was not observed. These results suggest that the graphene has no defects.

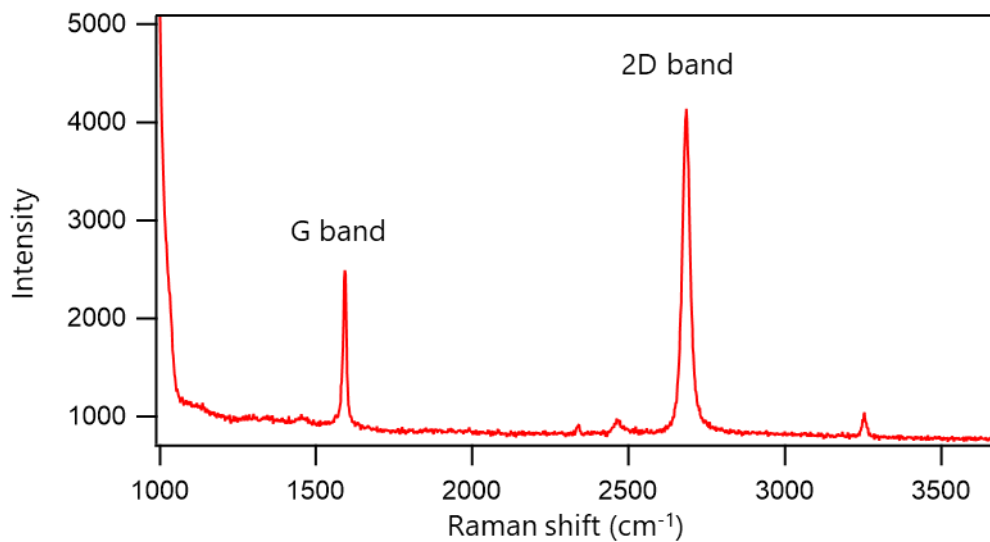


Fig 2.7. Raman spectrum of graphene field-effect transistor.

2.5 Summary

GFET for biosensor was successfully fabricated. Using CVD graphene, 32 GFET array was fabricated. These transfer characteristics show the ambipolar characteristics. In Raman measurements, D peak was not observed. It indicates that our graphene of GFET is homogeneous and high quality. Therefore, high sensitivity will be expected.

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3 Detection of SARS-CoV-2 virus

3.1 Introduction for detection of SARS-CoV-2

Here, we measured the whole SARS-CoV-2 using a GFET array integrated with a portable microchannel measurement system. The array was composed of multiple GFETs with SARS-CoV-2 spike antibodies. Multiple non-modified GFETs were also integrated onto the same substrate, and used as reference sensors. As the GFET characteristics were diverse due to the fabrication process variation, we calculated their means to cancel out the variation and improve the measurement reliability. We also calculated the difference in GFET responses between the Ab-modified and non-modified GFETs to suppress noise from the physisorption of the virus and baseline drifts. Furthermore, we implemented a microchannel over the GFET array to change buffer ionic strengths. The virus is effectively bound to receptors in a physiological ionic strength¹, whereas the GFET response becomes stronger in a lower ionic strength², indicating a trade-off between binding and detection. Therefore, we incubated the virus in a higher ionic strength solution and subsequently measured the virus in a lower ionic strength solution. The portable microchannel measurement system facilitated buffer exchange without manual pipetting, reducing perturbations by the exchange and improving reproducibility. Following the virus measurements, we observed the GFET array by scanning electron microscopy (SEM) to confirm whether the signal was responsible for the virus binding. Our custom-made portable microchannel measurement system enables one to measure the virus outside of the laboratory including at home.

3.2 Purpose

As described in Section 2.2, we aim to develop a more accurate measurement system. In this section, SARS-CoV-2 charge is detected using GFET. Moreover, Ab modification is evaluated for accurate measurement. Also, SARS-CoV-2 particles are observed by SEM.

3.3 Detection results of SARS-CoV-2

3.3.1 Antibody modification

The prepared GFET array was modified with the Ab using the following procedure. GFET channel size was 10 μm length and 100 μm width as shown in Fig. 3.1(a). Graphene was placed onto the channel between the source and drain electrodes. To divide the Ab-modified GFETs and the non-modified GFETs individually, silicone rubber was used to separate the GFET array into two regions: #0-#14 and #19-#31 as shown in Fig. 3.1(b). The GFETs of #15-#18 located under the rubber were not functionalized and not used in the analysis. The Ab modification method is described below and illustrated in Fig. 3.1(c). 1-Pyrenebutyric acid N-hydroxy-succinimide ester (PBASE) was dissolved in 2-methoxyethanol at a concentration of 5 mM. PBASE solution was subsequently dropped and incubated on the GFETs (#0-#14) at room temperature for 1 hour. On the other hand, the GFETs (#19-#31) were incubated with 2-methoxyethanol alone, without PBASE. Following the PBASE modification, the Ab was immobilized as follows. The Ab at a concentration of 40 $\mu\text{g/mL}$ in 30 mM PBS was incubated on the PBASE-modified GFETs (#0-#14) overnight at room temperature. The Ab was immobilized on the graphene surface through the process. On the other hand, the GFETs (#19-#31) were incubated with 30 mM PBS alone, resulting in the non-modified GFETs. After washing, all GFETs were treated with 1 mM ethanolamine-HCl for 30 min at room temperature to inactivate unbound PBASE. After the Ab modification, the rubber with two chambers was removed. Finally, the GFET array was treated with 0.01% tween 20 in 1.5 mM PBS at room temperature for 1 hour to block non-specific adsorption.

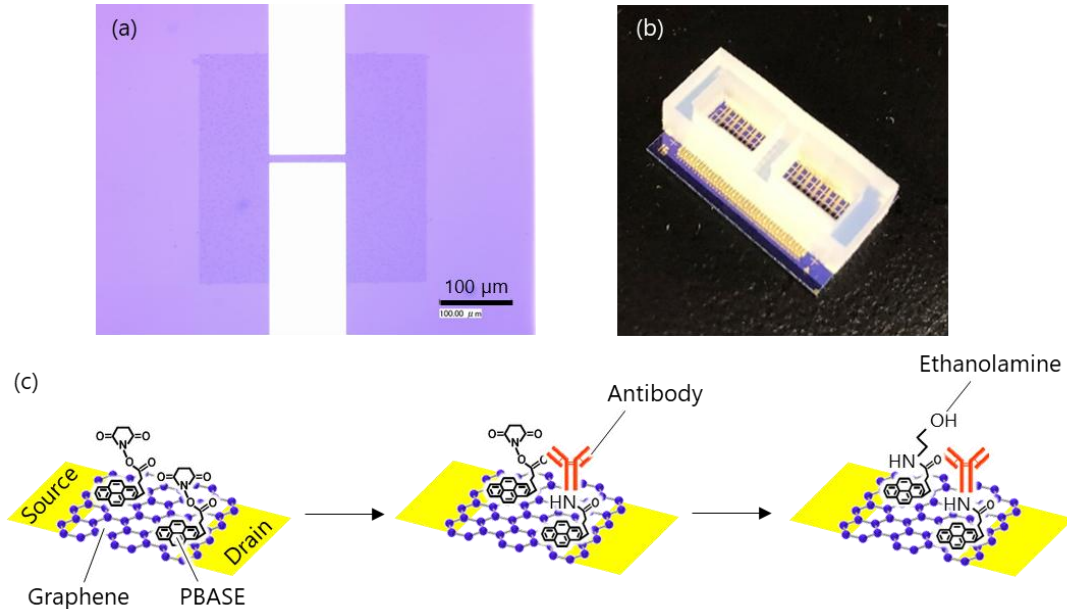


Fig 3.1. (a) Optical image of a typical graphene field-effect transistor (GFET) before the modification. (b) Optical image of silicone substrate with silicone rubber for dividing GFET area. (c) Illustration of antibody modification method.

3.3.2 Virus inactivation

For safe experiments, SARS-CoV-2 (JPN/TY/KW-521) was inactivated by using β -propiolactone, which is widely used for the inactivation of viruses³. The inactivated viruses were then filtrated and purified by ultracentrifugation. The resultant virus concentration was 1×10^9 FFU/mL. Prior to the FET experiments, the affinity between the inactivated virus and the spike Ab was confirmed by using bio-layer interferometry. The virus solution was diluted with 0.01% tween 20 in 1.5 mM PBS and applied to the FET measurement.

3.3.3 Debye length

For a better understanding of the measurement procedure, the definition of Debye length is quite important. The Debye length is defined as below.

$$\delta = \sqrt{\epsilon_l \epsilon_0 k_B T / 2 I N_A e^2}$$

where δ is the Debye length, ϵ_l is the relative permittivity of the electrolyte, ϵ_0 is the vacuum permittivity, k_B is the Boltzmann constant, T is the absolute temperature, I is the ionic strength of electrolyte, N_A is the Avogadro constant, and e is the elementary charge. Also, I is defined as the formula below.

$$I = \frac{1}{2} \sum_i c_i z_i^2$$

where c_i is molar concentration of ion species i , and z_i is valence of ion species i . Debye length becomes longer by using the electrolyte at a lower concentration because I is in the denominator in the above formula. As previously described, two kinds of solutions were used in this measurement. 150 mM PBS at a high concentration was used because virus and receptor can well bind at high 150 mM PBS. But, in 150 mM PBS, Debye length is approximately 0.7 nm and it is shorter than the receptor. Consequently, viral charge is screened and cannot be detected by GFET. Thus, 1.5 mM PBS was exchanged to extend Debye length to approximately 7 nm. This Debye length enables to detect a part of viral charge.

3.3.4 Procedure of virus detection

All electrical measurements were performed in either 0.01% tween 20 in 1.5 mM PBS or 0.01% tween 20 in 150 mM PBS. While sweeping V_{GS} from 0 to 500 mV, I_{SD} was measured for each GFET with the custom-built measurement equipment. The V_{GS} at the minimum I_{SD} is known as the charge neutrality point or DP. The value of DP for each GFET was calculated by polynomial fitting, and used to evaluate the GFET response. I_{SD} of DP changes when g_m and/or the resistance change. Therefore, the measurement at a constant I_{SD} could not be used. During the experiments, a few GFETs were disconnected and those were not used for the analysis.

3.3.5 Microchannel measurement system

A microchannel was set on the GFET array, as shown in Fig. 3.2(a). Fig. 3.2(b) provides an overview of the portable microchannel measurement system. The microchannel comprises four through holes, where the side two holes function as an inlet and outlet for 1.5 mM PBS. 1.5 mM PBS flowed through the channel at a constant rate of 7 $\mu\text{L}/\text{sec}$ by using micropumps. The central hole is for inserting a Ag/AgCl electrode. In this study, the Ag/AgCl electrode was used as a reference electrode without KCl salt bridge. In the present study, only 1.5 mM PBS was used during the measurement for the viral detection. Therefore, the potential disturbance does not occur by the solution exchange. The hole beside the inlet is for injecting the virus solution or 150 mM PBS. The cavity volume is approximately 100 μL .

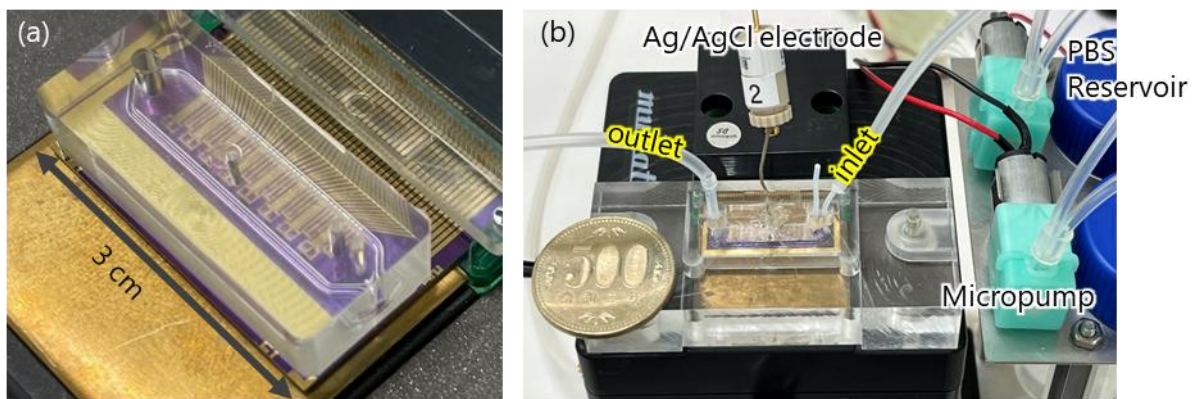


Fig. 3.2. (a) Photo of microchannel over graphene field-effect transistor array. (b) Photo of portable microchannel measurement system during electrical measurements.

3.3.6 Measurement results of antibody charge

We confirmed whether PBASE and the Ab were modified on the graphene surface by measuring the shift values of DP. Figs. 3.3(a) and (b) show that, before the modification, the means and standard deviations of DP of the GFETs (#0-#14) and those of the GFETs (#19-#31) were 289 ± 36 mV and 361 ± 52 mV, respectively, indicating deviation between GFETs due to the fabrication process variation. After the PBASE modification, the DP values of the modified GFETs increased to 310 ± 40 mV, as provided in Figs 3.3(c) and (d). The positive shift may be attributed to the pyrenyl group in PBASE, which agrees with previous reports. In contrast, the DP values of the non-modified GFETs were negatively shifted to 255 ± 23 mV. The negative shift was attributed to the 2-methoxyethanol incubation. 2-methoxyethanol may dissolve contaminants such as PMMA residue, known as a p-type dopant, thus resulting in the downshift of the DP values. Consequently, the incubation of PBASE in 2-methoxyethanol induced the competing response of up- and downshifts of the DP values, and the data indicates the upshifts were predominant. After the Ab immobilization, the DP values of the modified GFETs (#0-#14) decreased to 256 ± 24 mV, as shown in Fig. 3.3(e)(f). The negative shift may be attributed to the Ab. On the other hand, the DP values of the non-modified GFETs (#19-#31) remained almost unchanged after the 30 mM PBS incubation.

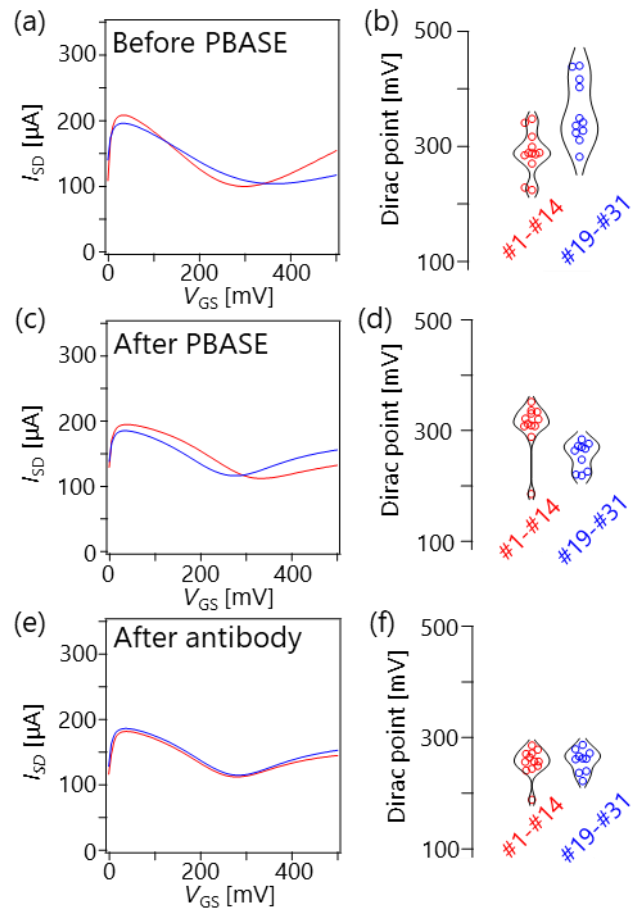


Fig. 3.3. (a)(c)(e) mean I_{SD} - V_{GS} plots (a) before PBASE, (c) after PBASE, and (e) after antibody (Ab) modification. (b)(d)(f) Violin plots of DP values (b) before PBASE, (d) after PBASE, and (f) after Ab modification. Red and blue colors represent the graphene field-effect transistors (#1-#14) and those (#19-#31), respectively.

3.3.7 Measurement results of SARS-CoV-2

Using the fabricated devices, we monitored responses to SARS-CoV-2 at a concentration of 1×10^8 FFU/mL. As previously described in Section 3.3.3, to enhance the detection efficiency, buffer exchange was implemented during the measurements. Virus binds well in 150 mM PBS. In contrast, Debye length in 1.5 mM PBS is estimated to be approximately 7 nm⁴, which is 10 times as long as that in 150 mM PBS, thus improving the transducing efficiency. Consequently, there is a trade-off between the binding and transducing efficiency. To mitigate the trade-off, we used 150 mM PBS for the virus binding and 1.5 mM PBS for the electrical measurements. The buffer exchange was conducted by using the microchannel system. Fig. 3.4(a) displays the time series of DP for the virus measurement while changing the ionic strengths of the buffers. The measurement includes seven phases denoted as (i) to (vii). During the measurements, the blank sample, i.e. 150 mM PBS with no virus, was introduced twice at the (ii) and (iv) phases to evaluate how much the buffer exchange process affected the DP values. Prior to the (i) phase measurement, the device was treated with 0.01% tween 20 for blocking, which resulted in a gap in the initial DP values between the Ab-modified and non-modified GFETs. In the (i) phase, the GFETs were measured while keeping 1.5 mM PBS flowing. Following the (i) phase, 1.5 mM PBS was completely drained and 150 mM PBS was subsequently injected and measured for 1 hour in the (ii) phase. In the (iii) phase, the GFET array was washed with 1.5 mM PBS flowing and measured in 1.5 mM PBS, as in the (i) phase. The (iv) and (v) phases followed the same protocol as in the (ii) and (iii) phases, respectively. Following, in the (vi) phase, SARS-CoV-2 at a concentration of 1×10^8 FFU/mL in 150 mM was injected and incubated for 1 hour. Finally, in the (vii) phase, the virus solution was exchanged to 1.5 mM PBS, in which unbound viruses were washed away, and then the device was measured for a while. It is noted that in the (ii), (iv), and (vi) phases, where 150 mM PBS was used, the DP values were not calculated because those were shifted downwards and out of the V_{GS} sweep range, although the electrical measurements were performed during these phases.

To analyze the response of each GFET against the sample, we used DP values at the endpoints of the measurement phases ((i), (iii), (v) and (vii)) and defined ΔV_{DP} , which is a change against the samples, i.e., the difference between before and after the sample injection phases ((ii), (iv) and (vi)). Fig. 3(b)-(d) represents the ΔV_{DP} for the first and second blank samples, and for the virus, respectively. According to Fig. 3.4(b) and (c), the means of ΔV_{DP} of the Ab-modified GFETs (ΔV_{DP}^w) for the first and second blank samples were -1.1 mV and 0.2 mV, respectively. In contrast, the means of ΔV_{DP} of the non-modified GFETs ($\Delta V_{DP}^{w/o}$) were -11.2 mV and -6.8 mV, respectively. The reason for the larger absolute value of $\Delta V_{DP}^{w/o}$ than ΔV_{DP}^w is yet to be understood and shall be studied in the future.

Herein, we derived the signal S as the difference between ΔV_{DP}^w and $\Delta V_{DP}^{w/o}$, namely $S = \Delta V_{DP}^w - \Delta V_{DP}^{w/o}$, to cancel out noises due to baseline drifts and physisorption of substances. S for the first and the second blank samples were determined as 10.1 mV and 7.0 mV, respectively. Consequently, we

supposed the mean S for the blank sample (S_0) was equal to 8.6 mV, which can be considered as a perturbation of the buffer exchange. For the results of the virus sample in Fig. 3.4(d), ΔV_{DP}^w and $\Delta V_{DP}^{w/o}$ were calculated as 19.3 mV and 2.8 mV, respectively. Both the ΔV_{DP}^w and $\Delta V_{DP}^{w/o}$ were considerably upshifted by the virus introduction, in contrast to the blank samples. The upshifts may be attributed to the negative charge of the virus⁵. In addition, the positive value of $\Delta V_{DP}^{w/o}$ implies the presence of physisorbed viruses. Based on the obtained ΔV_{DP}^w and $\Delta V_{DP}^{w/o}$, S was calculated as 16.5 mV, in which the response due to the physisorption of the virus was canceled out by subtraction. In this study, the important parameter in the preset study is not

the fluctuation of the baseline, but the amount of S . S of the first and the second blank samples is the low value of 10.1 mV and 7.0 mV, respectively. In contrast, when the SARS-CoV-2 was introduced to the microchannel, S is increased as high as 16.5 mV. Thus, it is said that S was increased by the binding of SARS-CoV-2 to Ab selectively. It is noted that the S of 16.5 mV should include S_0 , which is the perturbation of the solution exchange process. Consequently, the net S against the virus injection was estimated to be $S - S_0 = 7.9$ mV. It is important to note that the size of the viruses approximately 100 nm⁶ was much larger compared to the Debye length of about 7 nm in 1.5 mM PBS. Therefore, the charges of the underside of the virus may be accountable for the net S .

The detection of SARS-CoV-2 in saliva by GFET is the future target. SARS-CoV-2 in saliva have been tried to detect by using GFET modified with SARS-CoV-2 spike RBD Ab and the reference GFET modified with influenza Ab. GFET modified with influenza Ab is the more accurate reference than non-modified GFET used in the present study. It was confirmed that saliva does not affect the baseline because DP did not shift before and after the introduction of the saliva.

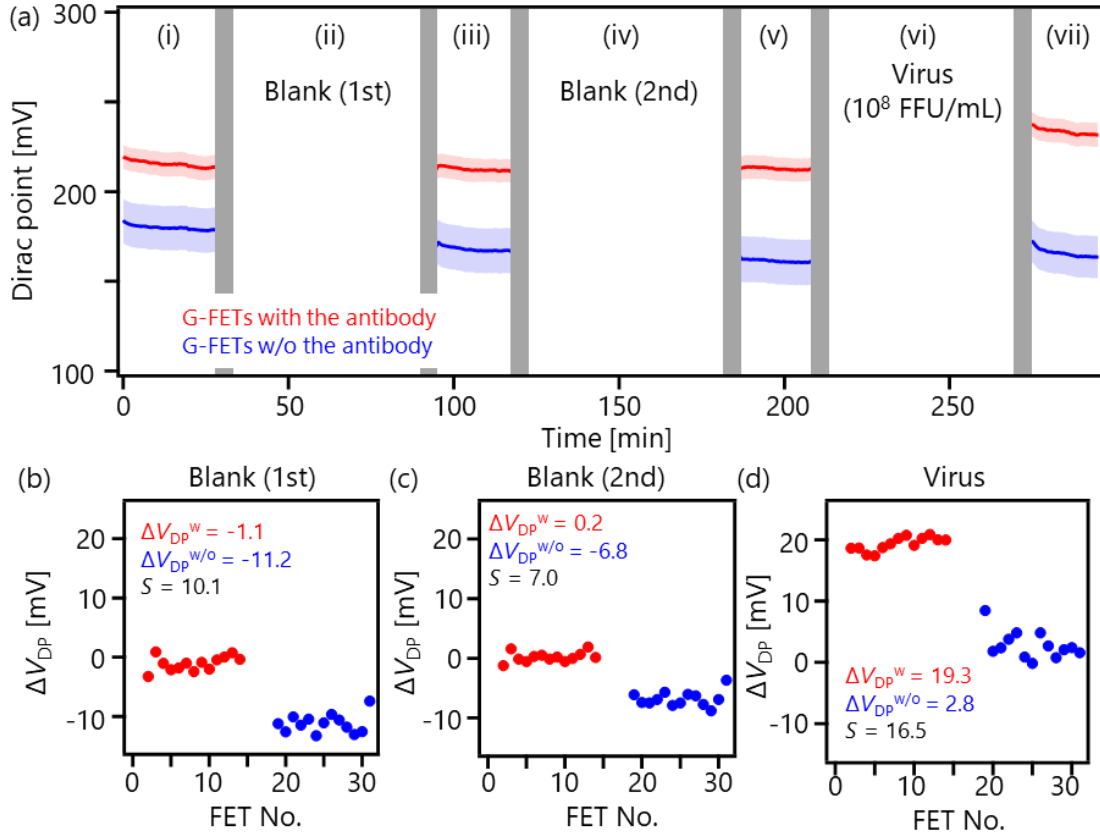
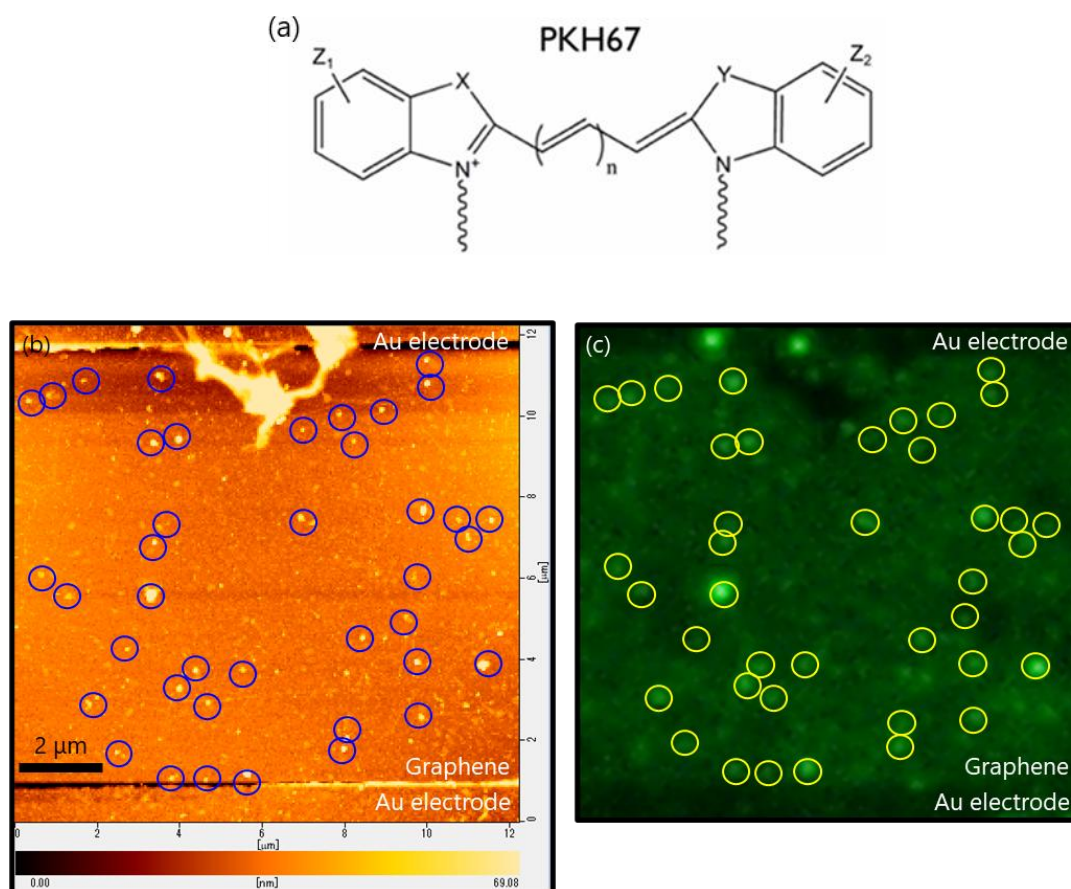


Fig 3.4. (a) Time series of Dirac point values for virus measurement including (i) - (vii) phases. In the (ii) and (iv) phases, 150 mM PBS was injected as a blank sample, and in the (vi) phase, the virus at a concentration of 1×10^8 FFU/mL was injected. The mean DP values of the graphene field-effect transistors (GFET) with the antibody (Ab) and those of the GFETs without the Ab were plotted as red and blue lines, respectively. The shades represent their standard deviation. The gray bars represent the period when the electrical measurements were not performed properly due to the airflow through the microchannel. (b)(c)(d) ΔV_{DP} for the first and second blank samples, and for the virus, respectively.

3.3.8 Identification of virus particles using SEM-observation

First of all, the virus was observed by atomic force microscope (AFM) and fluorescence observation. Viral particles were observed using AFM with heights ranging from 20 to 70 nm and circled in blue. On the other hand, using fluorescence microscopy, the viral particles were labeled with the fluorescent dye PKH 67⁷ as shown in Fig. 3.5(a). The carbon chains of PKH intercalate into the viral envelope. PKH emits fluorescence when excited by 520 nm light. Fig. 3.5(b) represents the result of fluorescence observation. Viruses are fluorescent and circled in yellow line. Fluorescence imaging and AFM imaging were performed at the same location of the FET as shown in Fig. 3.5(c). Moreover, viral particles were observed by SEM at the same location as shown in Fig. 3.5(d). The AFM-observed particles coincide in position with the SEM particles that appear bright outside and dark inside. The edges of spherical particles appear bright due to an edge effect. At the edges, the effective area contributing to secondary-electron emission becomes larger, resulting in a relatively higher emission yield⁸. As a result, the particles that appeared bright at the edges and dark at the center in the SEM images were identified as viruses.



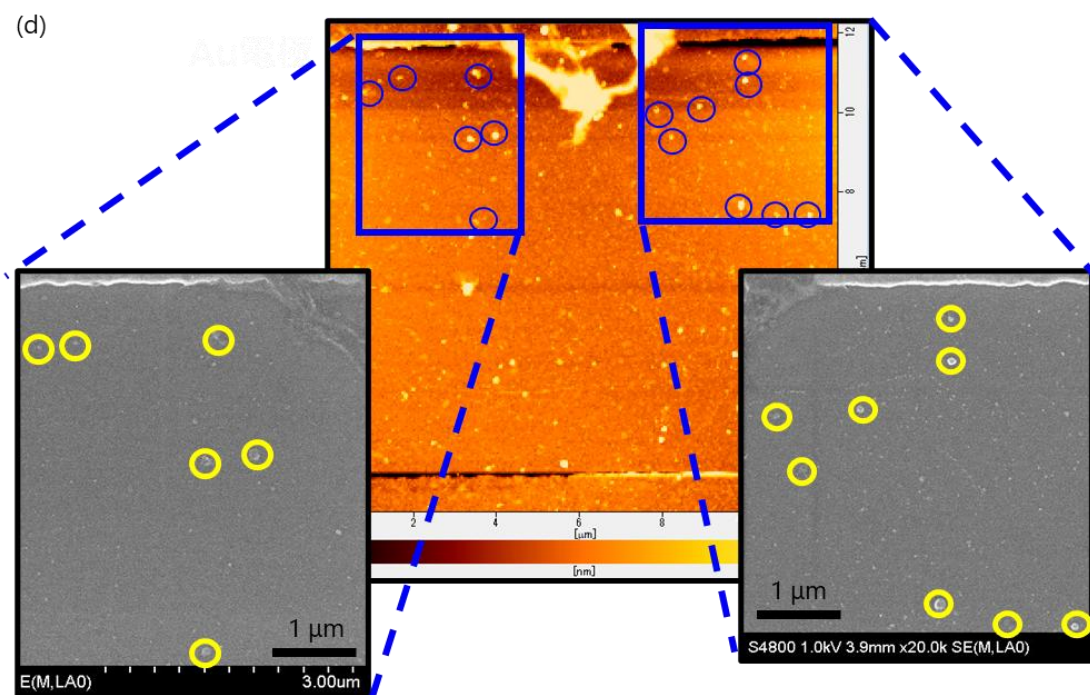


Fig. 3.5. (a) Structure of PKH 67. (b) AFM image. Height of viral particles is ~ 70 nm. (c) fluorescence microscopy image of virus. Bright spots indicate presence of viruses. (d) Viral particles observed by SEM at the same location of AFM image.

3.3.9 Surface observation using SEM

To confirm whether the signal was responsible for the virus binding, the GFET array after the electrical measurements was observed by using SEM (S-4800, Hitachi High-Tech). Figs. 3.6(a) and (b) are typical SEM images of a part of the Ab-modified GFET channel. The arrows in Fig. 3.6(a) indicate the positions of the viruses. As shown in Fig. 3.6(b), the size of the virus was approximately 100 nm, which aligns with previously reported values. For comparison, a typical SEM image of a GFET without the Ab modification is presented in Fig. 3.6(c), showing fewer adsorbed viruses. The numbers of the viruses in each GFET channel are summarized in Fig. 3.6(d). In the Ab-modified GFETs, the number of the viruses ranged from 21 to 56, with a mean of 37. In contrast, the number of viruses ranged from 1 to 9, with a mean of 4, in the non-modified GFETs. The results indicate that the adsorption of viruses on the Ab-modified graphene surface was roughly 10 times more than on the non-modified surface, as shown in Fig. 3.6(d). Although the SEM analysis is unable to distinguish between specifically adsorbed and physisorbed viruses, the SEM results support that the viruses were preferentially adsorbed onto the Ab. Figs. 3.6(a)-(c) is the SEM image after the washing process of the viral measurement. Even after the washing process, SARS-CoV-2 selectively binding to Ab can be observed. Fig. 3.6(e) plots ΔV_{DP} in Fig. 3.4(d) as a function of the number of viruses. The plot confirms that larger ΔV_{DP}^w was attributed to increased virus adsorption. Additionally, Fig. 3.6(e) also suggests the presence of physisorbed viruses on the non-modified graphene surface, which may be responsible for the positive values of $\Delta V_{DP}^{w/o}$ against the virus injection. Based on these results, it can be concluded that our GFET devices responded to the virus specifically adsorbed onto the Ab.

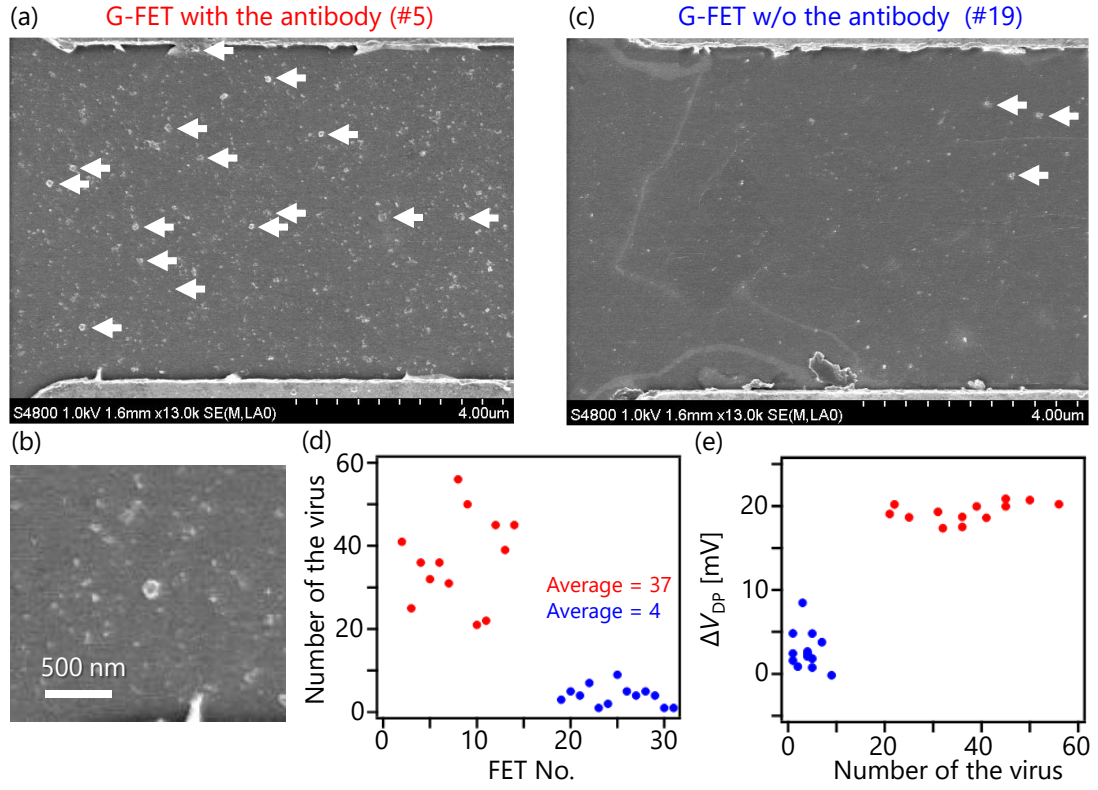


Fig. 3.6. (a) SEM image of the graphene field-effect transistor (GFET) with the antibody (Ab) (#5). (b) The enlarged SEM image of (a). (c) SEM image of the GFET without the Ab (#19). The arrows in (a) and (c) indicate the position of the viruses. (d) Number of adsorbed viruses for each GFET channel. (e) ΔV_{DP} as a function of the number of adsorbed viruses. Red and blue colors represent the GFETs with the Ab and those without the Ab.

3.4 Summary

We have developed GFET arrays with the portable microchannel measurement system for SARS-CoV-2 detection. Two types of GFETs, i.e., with and without the Ab modification were integrated on a single substrate. Based on the configuration, we aimed to minimize noise due to baseline drifts and physisorption of substances, by computing the difference between the mean DP values of the Ab-modified and the non-modified GFETs. Upon introducing the virus at a concentration of 1×10^8 FFU/mL to the GFET array, the virus response was obtained as the net DP shift of 7.9 mV. The response was verified through SEM observation. The SEM analysis demonstrated that the number of viruses on the Ab-modified GFETs was approximately 10 times greater than that on the non-modified GFETs, indicating the selective binding of the virus to the Ab. Therefore, the SEM analysis supported the FET-response against the virus. Furthermore, our measurement system offers several advantages, such as a simple protocol and short measurement time, and portability, when compared to conventional PCR tests. Owing to these advantages, the presented GFET biosensor system has the potential to contribute to the development of reliable point-of-care rapid test technology for pandemic prevention.

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4 Relationship between surface charge density of graphene and sensitivity of GFET

4.1 Purpose

To enhance the sensitivity of GFET, the relationship between the surface charge density of graphene and the sensitivity was theoretically analyzed. By combining the formula of Poisson equation and Boltzmann distribution, relationship between the voltage from reference electrode V_1 and the surface charge density of graphene Q_s/eS was derived. The sensitivity of GFET was obtained by differentiating V_1 with respect to Q_s/eS . The sensitivity of each electrolyte such as PBS and acetate buffer was evaluated.

4.2 Deviation and explanation of sensitivity equation

To enhance the sensitivity of GFET, the Graham equation was used as a reference in our study. The Grahame equation which shows the relationship between the surface potential and the surface charge density is shown below.

$$\sigma = \sqrt{8k_B T \varepsilon \varepsilon_0 c_0} \sinh\left(\frac{zq\Psi_0}{2k_B T}\right) \quad (1)$$

where σ represents the charge density of a unit area, k_B represents the Boltzmann constant, ε represents a relative permittivity, ε_0 represents the vacuum permittivity, c_0 represents the bulk ionic strength, z represents the valency of the electrolyte, q represents the elementary charge, Ψ_0 represents surface potential. From the Grahame equation (1), the sensitivity considering the concentration of electrolyte and the pH can be approximated as¹

$$\frac{d\Psi_0}{d\sigma_{ana}} \sim \frac{1}{\cosh\left(\frac{zq\Psi_0}{2k_B T}\right)}. \quad (2)$$

From this equation (2), the sensitivity could be maximized when $\cosh\left(\frac{zq\Psi_0}{2k_B T}\right)$ becomes near 1, i.e., Ψ_0

becomes near zero and/or the surface charge density calculated from equation (1) becomes near zero. This equation can be applied only to monovalent ion in electrolyte.

Using these equations (Eq. 1 and Eq. 2) as reference, we derived the relationship between the surface charge density Q_s/eS and voltage of electrolyte V_1 (Eq. 3)². This equation was derived from the Poisson equation (Eq. 4) and the Boltzmann distribution (Eq. 5). Eq.3 can be applied to multivalent ion in electrolyte such as PBS and acetate buffer which will be used in our experiments.

$$\frac{Q_S}{S} = \pm \sqrt{2k_B T \varepsilon_l \varepsilon_0 \sum_n \rho_n \left(e^{\frac{neV_l}{k_B T}} - 1 \right)} \quad (3)$$

$$\rho(z) = -\varepsilon \frac{d^2 \Phi(z)}{dz^2} \quad (4)$$

$$\rho(z) = \sum_n ne\rho_n e^{-\frac{ne(\Phi(z)-\Phi_1)}{k_B T}} \quad (5)$$

where Q_S represents the surface charge density of graphene, S represents the area of the GFET, k_B represents the Boltzmann constant, T represents the absolute temperature, ε_l represents a relative permittivity, ε_0 represents the vacuum permittivity, n represents the ion valency, ρ_n represents the quantity of ions in 1 nm^3 , V_l represents the electrolyte voltage, $\Phi(z)$ represents the potential at distance z from the graphene surface, and Φ_1 represents the potential far from the graphene surface and reference electrode. V_l is assumed to be the voltage applied from a reference electrode because the actual surface potential of graphene is unknown.

4.3 Sensitivity calculated from sensitivity equation

4.3.1 Calculation of ion concentrations for two kinds of buffer solutions

Ion concentrations in 1.5 mM PBS (pH 7) and 1 mM acetate buffer (pH 4) were calculated from the measured pH values using the Henderson–Hasselbalch equation. The ion concentrations in the PBS were 1.53 mM Na^+ , 0.0415 mM K^+ , 1.40 mM Cl^- , 0.0554 mM HPO_4^{2-} , and 0.0403 mM H_2PO_4^- . In the acetate buffer, the ion concentrations were 0.075 mM Na^+ , 0.154 mM H^+ , and 0.229 mM CH_3COO^- .

4.3.2 Differences of graphs based on electrolyte

The blue lines in Figs. 4.1(a) and (b) show the relationship between Q_s/eS and V_1 from Eq. 3 for PBS and the acetate buffer, respectively. The horizontal, left vertical, and right vertical axes represent Q_s/eS , V_1 , and sensitivity, respectively. Sensitivity is obtained by differentiating V_1 by Q_s/eS and is illustrated by the red line in Figs. 4.1(a) and (b). The sensitivity in acetate buffer was higher than that of PBS due to the lower ionic strength of the acetate buffer. The highest sensitivities of GFET in both buffers were observed when the surface charge density of graphene was approximately zero.

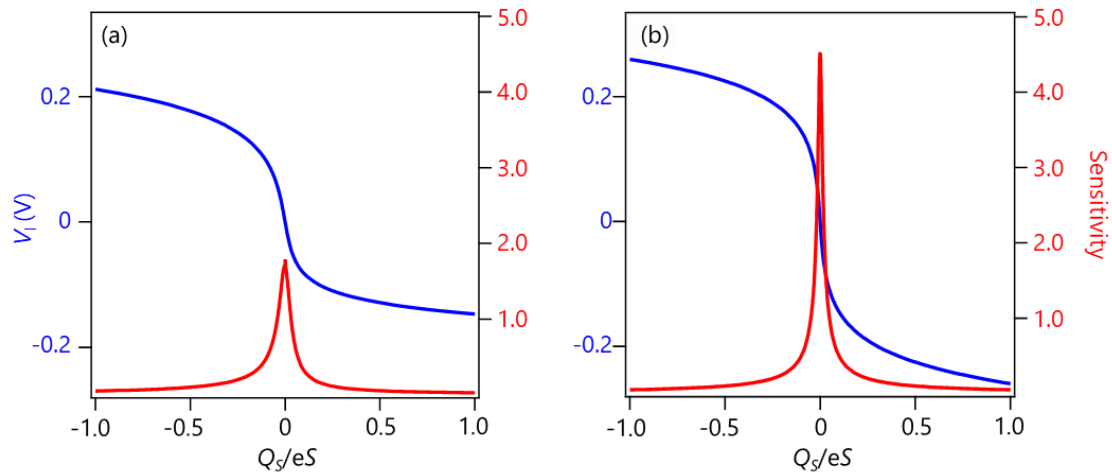


Fig. 4.1. The blue line indicates the relationship between the surface charge density of graphene (Q_s/eS) and electrolyte voltage (V_1) from Eq. 3, and the red line indicates the sensitivity of graphene field-effect transistor. (a) Calculated for 1.5 mM phosphate-buffered saline. (b) Calculated for 1 mM acetate buffer.

4.4 Summary

As a result, the highest sensitivity of GFET using PBS and acetate buffer represents at the surface charge density of zero of graphene surface. Moreover, the sensitivity of GFET using acetate buffer is higher than PBS due to the lower concentration of ions.

References

- 1) Mihir Gupta, Sybren Santermans, Geert Hellings, Liesbet Lagae, Koen Martens, and Willem Van Roy, IEEE SENSORS JOURNAL **21**, NO. 4, 4143 (2021).
- 2) K. Yamamoto, N. Sato, K. Sakano, M. Yano, E. Ohnishi, T. Ono, Y. Kanai, S. Ushiba, N. Miyakawa, S. Tani, M. Kimura, Y. Watanabe, K. Inoue, H. Tanaka, and K. Matsumoto, Jpn. J. Appl. Phys. **63** [3], 03SP14 (2024).

5 Enhancing sensitivity of biosensor by surface potential control with PLL

5.1 Introduction of antibody functionalized GFET with PLL

In this study, the ζ potential modulator which is PLL was introduced into GFET to enhance sensitivity. PLL is a well-known coating agent that improves cell adherence on hydrophobic plastic surfaces. As PLL can coat hydrophobic materials, we applied it to graphene, which is also hydrophobic. Fig. 5.1 represents a schematic illustration of GFET with PLL. To get the optimum PLL concentration, GFET modified with the different concentrations of PLL was used to detect SARS-CoV-2. PLL concentration was changed from 0.5 ng/mL to 5 mg/mL. As a result, maximum DP shift due to SARS-CoV-2 was obtained when GFET was modified with 50 $\mu\text{g/mL}$ PLL. Thus, 50 $\mu\text{g/mL}$ PLL was used all through this study. When the surface charge density of the graphene is almost zero, GFET shows the highest sensitivity as shown in Fig. 4.1. Owing to O^{2-} and/or $-\text{OH}$, $-\text{COOH}$ on the graphene surface, however, it is said that GFET have the negative ζ potential of $\sim -45 \text{ mV}^1$ in pure water at room temperature and it is difficult to get the higher sensitivity as it is. To get higher sensitivity, we introduced PLL on GFET as the ζ potential modulator with the positive charge which can cancel out the negative charge of the graphene surface. This effect reduces the surface charge density near to zero. In addition, we installed the microchannel onto GFETs to remove the physical adsorption of the virus and the virus not bound to the Ab in the solution. Using the microchannel, quantitative washing becomes possible. And the reliability of the mean and the standard deviation of the DP become increased compared to the conventional manual pipetting process.

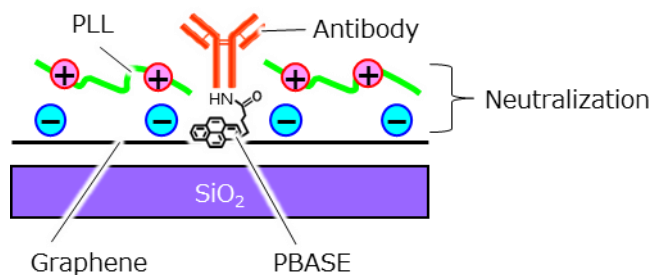


Fig. 5.1. Illustration of functionalized graphene field-effect transistor with poly-L-lysine.

5.2 Conformation of antibody and PLL modification

5.2.1 Procedure of antibody functionalized GFET with PLL

The method of Ab modification was described in Section 3.3.1. Fig. 5.2(a) represents the GFET used in this experiment. The channel length and width are 10 and 400 μm , respectively. Fig. 5.2(b) shows the schematic illustration of GFET procedure for the biosensing. The surface of GFET was modified by SARS-CoV-2 spike Ab or influenza H10N8 Hemagglutinin Ab via PBASE^{2,3,4}. To enhance the sensitivity of GFET biosensor, the negative charge at the graphene surface should be canceled out and the surface charge density of graphene should be as small as possible. For this purpose, PLL, which is a kind of cation polymer was added to GFET.

To compare the effects of PLL used as the ζ potential modulator, 4 kinds of GFETs were prepared as shown in Fig. 5.2(c). Also, to solve the effect of the physical adsorption of the virus and of the drift^{5,6}, the reference FETs were prepared. Two G-FETs were modified by SARS-CoV-2 spike Ab with (Area (i)) and without (Area (ii)) PLL. The other two GFETs were modified by influenza H10N8 Hemagglutinin Ab with (Area (iii)) and without (Area (iv)) PLL. 32 integrated GFETs are divided into 4 areas as shown in Fig. 5.2(c) by the silicon lubber pool. Each area is modified as follows:

- (i) Area of FETs modified with SARS-CoV-2 spike Ab and PLL.
- (ii) Area of FETs modified with SARS-CoV-2 spike Ab.
- (iii) Area of FETs modified with influenza H10N8 Hemagglutinin Ab and PLL.
- (iv) Area of FETs modified with influenza H10N8 Hemagglutinin Ab.

(iii) and (iv) were used as the reference FETs to remove the effect of physical adsorption of the viral charge and the drift.

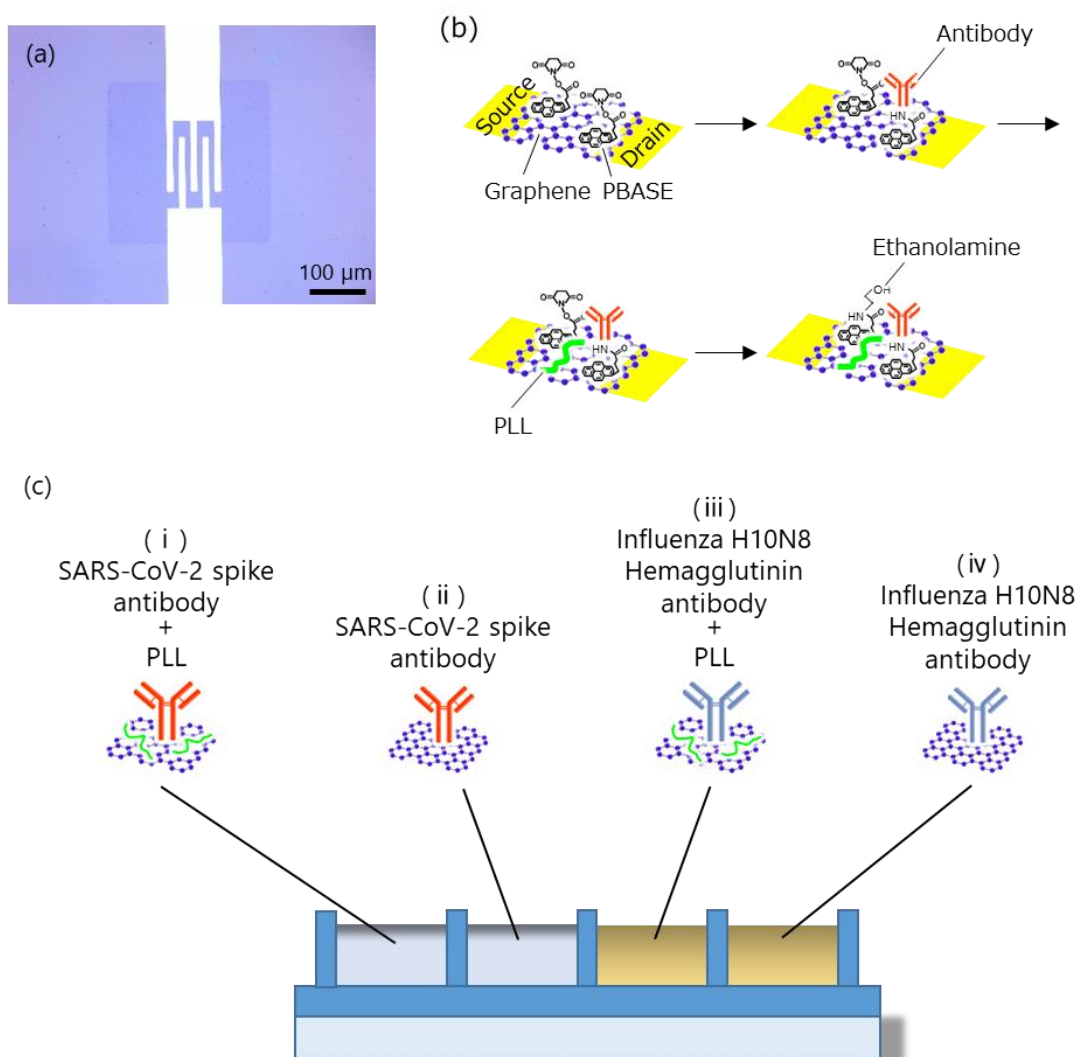


Fig. 5.2. (a) Optical image of expanded view of one graphene field-effect transistor (GFET). (b) Schematic illustration of antibody modification on GFETs. (c) 4 kinds of GFET were prepared on same substrate to compare effects of poly-L-lysine, and also to cancel out problems of physical adsorption of virus and that of drift.

5.2.2 Measurement of positive charges in PLL

Fig. 5.3 shows the transfer characteristics of GFET of area (i) in Fig. 5.2(c) before and after the introduction of the PLL. The PLL was introduced into GFET after modifying SARS-CoV-2 spike Ab as shown in Fig. 5.2(b). In Fig. 5.3, the horizontal axis is the V_{GS} and the vertical axis is the I_{SD} . In this experiment, V_{GS} was swept between 0 and 500 mV. The DP was measured by applying the top gate voltage when the microchannel was filled with 1.5 mM PBS. The blue line is the transfer characteristics before the introduction of PLL, and the red line after the introduction of PLL. In Fig. 5.3, the DP of GFET shifted in the negative direction after the introduction of PLL. This shift was caused by the positive charge of PLL which induce the negative charge in GFET. Thus, we could confirm that PLL was successfully introduced onto the graphene surface by this shift.

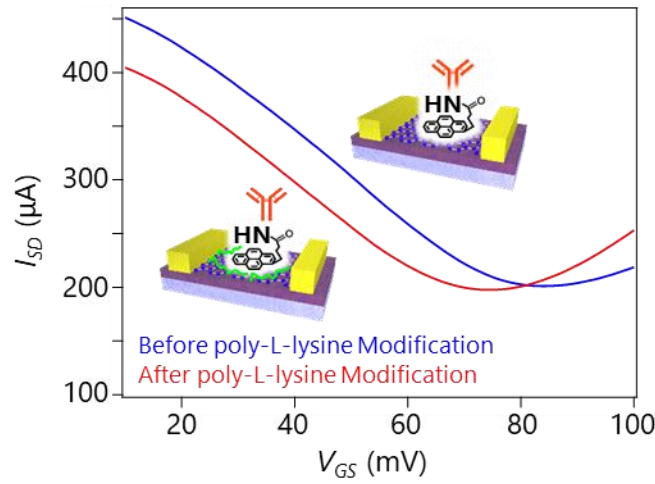


Fig. 5.3. Transfer characteristics of graphene field-effect transistor modified with SARS-CoV-2 spike antibody before and after introduction of poly-L-lysine.

5.3 Detection Results of SARS-CoV-2 using PBS

5.3.1 Procedure of virus detection

Viral measurements were performed under the following conditions: V_{GS} was swept from -100 to 400 mV in 1.5 mM PBS. During the V_{GS} sweep, I_{SD} was measured for each GFET using custom-built equipment.

5.3.2 Results of PLL effect

To confirm the enhancement effect of the sensitivity of GFET by the introduction of PLL, the sensitivity of GFET with and without PLL was compared as shown in Fig. 5.4. Fig. 5.4 shows the result of the detection of the inactivated SARS-CoV-2⁷ using GFET modified by SARS-CoV-2 spike Ab with (Area (i) in Fig. 5.2(c)) and without (Area (ii) in Fig. 5.2(c)) PLL. The blocking of GFET was performed using 0.01% Polyoxyethylene Sorbitan Monolaurate (Tween 20) before this measurement. The horizontal axis is the time and the vertical axis is the ΔV_{DP} . This graph is the calculated result by subtracting the mean of the DP of GFET without the PLL (Area (ii) in Fig. 5.2(c)) from that with PLL (Area (i) in Fig. 5.2(c)) to remove the effect of the physical adsorption of the virus and the drift. In Fig. 5.4, the measurement of the ΔV_{DP} starts at a low concentration of 1.5 mM PBS [1]. Then, a high concentration of 150 mM PBS without SARS-CoV-2 was introduced two times [2] [4] to the system to check whether there were no disturbance by the exchange of the solution. Then, 1×10^6 FFU/mL [6] and 1×10^7 FFU/mL [8] SARS-CoV-2 in 150 mM PBS were introduced into the system and the ΔV_{DP} shift was measured. If the viral charge enters within the Debye length, GFET can detect the negative charge of the SARS-CoV-2 virus⁸ and the ΔV_{DP} toward the positive direction. After the virus bound to the Ab sufficiently, 1.5 mM PBS was, then, introduced to the microchannel to wash away the free-floating virus in the solution and the physical adsorption of viruses on graphene, so that only the charge of the virus specifically bound to the Ab was detected [7] [9]. Before and after the exchange of the solution without SARS-CoV-2, i.e., the area [1] [3] [5], the ΔV_{DP} shift shows only small increase. The mean of these ΔV_{DP} is shown by the red dashed line in Fig. 5.4. The three times of the standard deviation, 3σ of the ΔV_{DP} in these areas of [1] [3] [5] is 2.3 mV, and is shown in the pink shadow. After the introduction of SARS-CoV-2 with concentrations of 1×10^6 and 1×10^7 FFU/mL, the large ΔV_{DP} shifts of 5 and 17 mV were observed, respectively. Because these values are beyond 3σ (2.3 mV), it is said that this response is due to the negative charge of SARS-CoV-2. Thus, these results imply that the sensitivity of GFET was improved by applying the PLL.

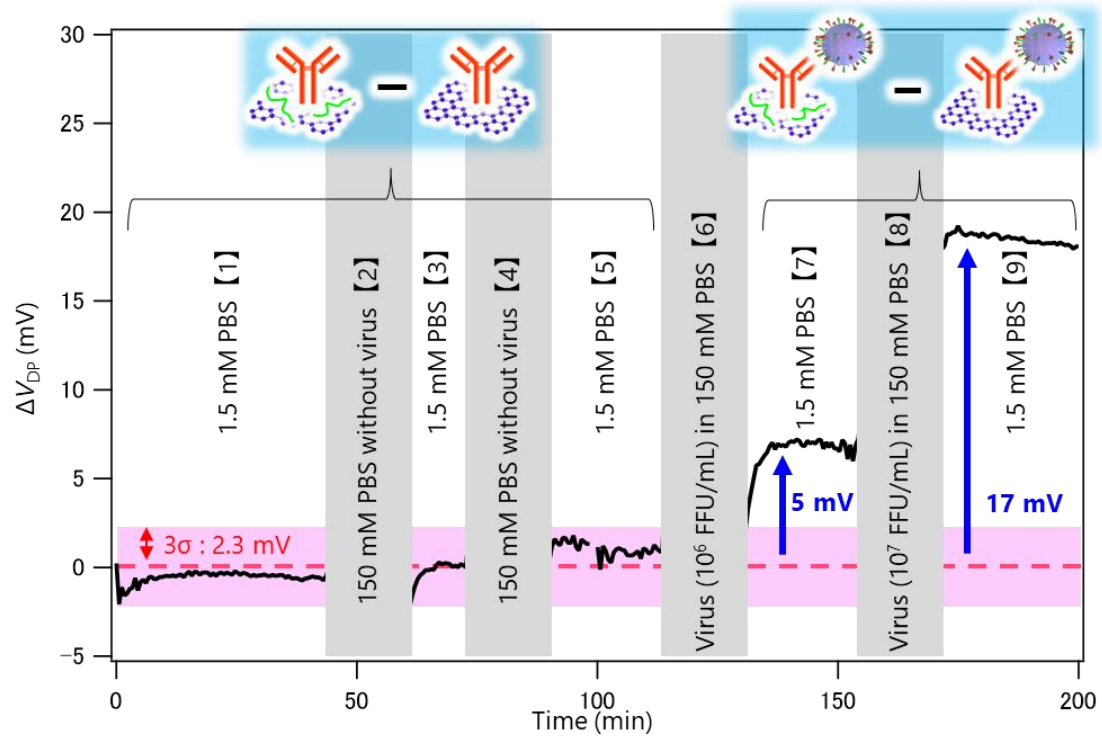


Fig. 5.4. Result of detection of SARS-CoV-2 using graphene field-effect transistor (GFET) modified by SARS-CoV-2 spike antibody with and without poly-L-lysine (PLL). Graph is calculated result by subtracting mean of Dirac point of GFET without PLL from that with PLL.

5.3.3 Detection of SARS-CoV-2 using GFET with PLL in PBS

Fig. 5.5 shows the experimental result of the detection of SARS-CoV-2 by using GFET modified with SARS-CoV-2 spike Ab and also with PLL (Area (i) in Fig. 5.2(c)). GFET modified with influenza H10N8 Hemagglutinin Ab with PLL (Area (iii) in Fig. 5.2(c)) was used as the reference FETs to remove the effect of the physical adsorption of the viral charge and the drift effect. The graph in Fig. 5.5 is the calculated results by subtracting the mean of the DP of GFET modified with influenza H10N8 Hemagglutinin Ab and PLL (Area (iii) in Fig. 5.2(c)) from that with SARS-CoV-2 spike Ab and PLL (Area (i) in Fig. 5.2(c)). The horizontal axis is the time and the vertical axis is the ΔV_{DP} . Two times of 150 mM PBS without the virus [11] [13] and two concentrations of SARS-CoV-2 in 150 mM PBS [15] [17] were introduced into the system. Subsequently, 1.5 mM PBS was introduced into the system [16] [18] to extend the Debye length about ten times for the detection of the viral charge as a shift of the DP. Before and after the exchange of the solution without SARS-CoV-2, i.e., the area [10] [12] [14], although the ΔV_{DP} shift shows only small increase, and the mean of these ΔV_{DP} is shown by the red dashed line in Fig. 5.5. Also, 3σ of the ΔV_{DP} in these areas of [10] [12] [14] is 4.9 mV, and is shown in the pink shadow. After the introduction of SARS-CoV-2 at a concentration of 1×10^6 FFU/mL, ΔV_{DP} was as small as ~ 1.3 mV [16] and it does not exceed 3σ and is considered to be under the noise level. After the introduction of SARS-CoV-2 at a concentration of 1×10^7 FFU/mL, however, ΔV_{DP} was as large as +10 mV [18] and it exceeds the 3σ . Therefore, it can be said that this response is originated from the negative charge of SARS-CoV-2 binding to the Ab, selectively. Moreover, it can be said that the sensitivity of GFET with PLL increased 10 times higher than that without PLL.

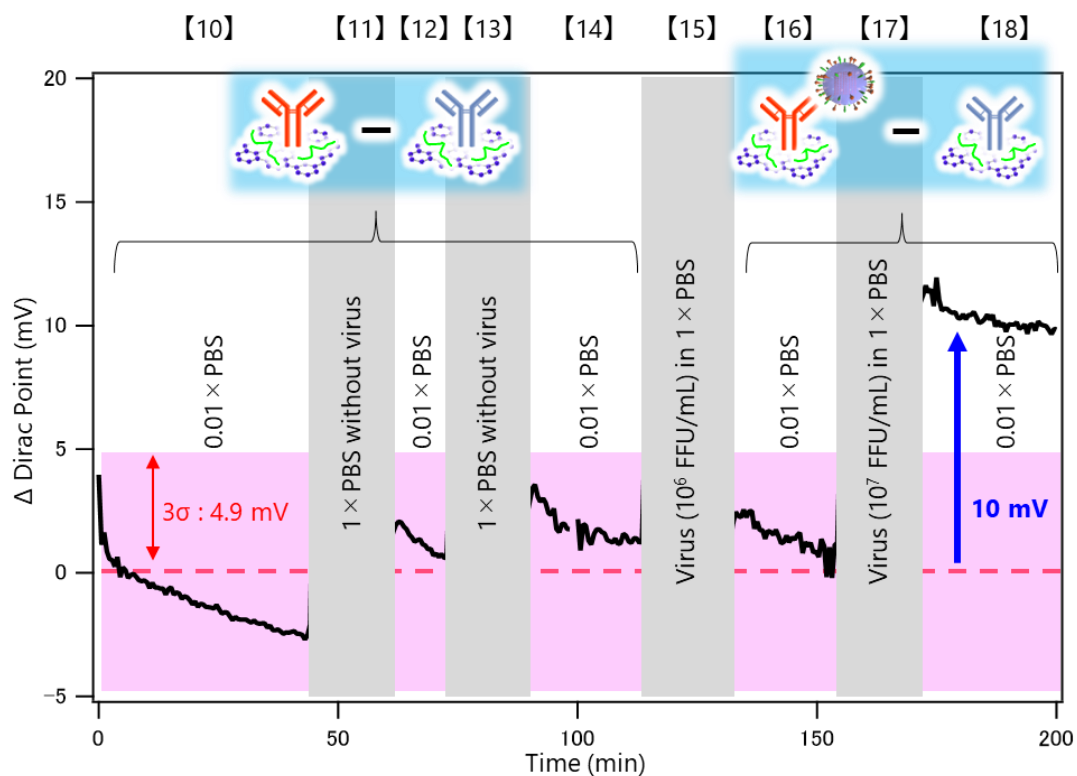


Fig. 5.5. Result of detection of SARS-CoV-2 using graphene field-effect transistor (GFET) modified by SARS-CoV-2 spike antibody (Ab) and by influenza H10N8 Hemagglutinin Ab. Both FETs are modified by poly-L-lysine. Graph is calculated result by subtracting mean of Dirac point of GFET modified by influenza H10N8 Hemagglutinin Ab from that by SARS-CoV-2 spike Ab.

5.3.4 Detection of SARS-CoV-2 using GFET without PLL in PBS

To confirm the enhancement effect of PLL, GFET “without PLL” was used to detect SARS-CoV-2. Fig. 5.6 shows the experimental result of the detection of SARS-CoV-2 by using GFET modified with SARS-CoV-2 spike Ab “without PLL” (Area (ii) in Fig. 5.2(c)). GFET modified with influenza H10N8 Hemagglutinin Ab “without PLL” (Area (iv) in Fig. 5.2(c)) was used as the reference FETs. Fig. 5.6 is the calculated results by subtracting the mean of the DP of GFET modified with influenza H10N8 Hemagglutinin Ab “without PLL” (Area (iv) in Fig. 5.2(c)) from that with SARS-CoV-2 spike Ab “without PLL” (Area (ii) in Fig. 5.2(c)). The horizontal axis is the time and the vertical axis is the ΔV_{DP} . Two times of 150 mM PBS without virus [20] [22] and two concentrations of SARS-CoV-2 in 150 mM PBS [24] [26] were introduced into the system. Subsequently, 1.5 mM PBS was introduced into the system to extend the Debye length about ten times for the detection of the viral charge [25] [27] as a shift of ΔV_{DP} . Before and after the exchange of the solution without SARS-CoV-2, i.e., the area [19] [21] [23], although the ΔV_{DP} shift shows only small increase, and the mean of these DP shifts is shown by the red dashed line in Fig. 5.6. Also, 3σ of the ΔV_{DP} in these areas of [19] [21] [23] is 5.7 mV, and is shown in the pink shadow. After the introduction of SARS-CoV-2 at concentrations of 1×10^6 and 1×10^7 FFU/mL, ΔV_{DP} does not exceed 3σ and is considered to be under the noise level [25] [27].

Comparing Fig. 5.4 with Figs. 5.5 and 5.6, the larger 3σ in Figs. 5.5 and 5.6 are considered to be due to the difference in the drift between GFETs. Fig. 5.4 is the result of subtracting the DP s of GFETs modified with the same type of Ab from each other, while Figs. 5.5 and 5.6 are the results of subtracting the DP of GFETs modified with the different types of antibodies. The different antibodies are thought to be responsible for the larger drifts in Figs. 5.5 and 5.6 compared to Fig. 5.4. The different environments on graphene often cause different properties such as drift.

Using PLL as shown in Fig. 5.5, SARS-CoV-2 at a concentration of 1×10^7 FFU/mL was clearly detected [18], but without PLL as shown in Fig. 5.6, SARS-CoV-2 at the same concentration could not be detected [27]. These results clearly show the enhancement of the sensitivity using PLL.

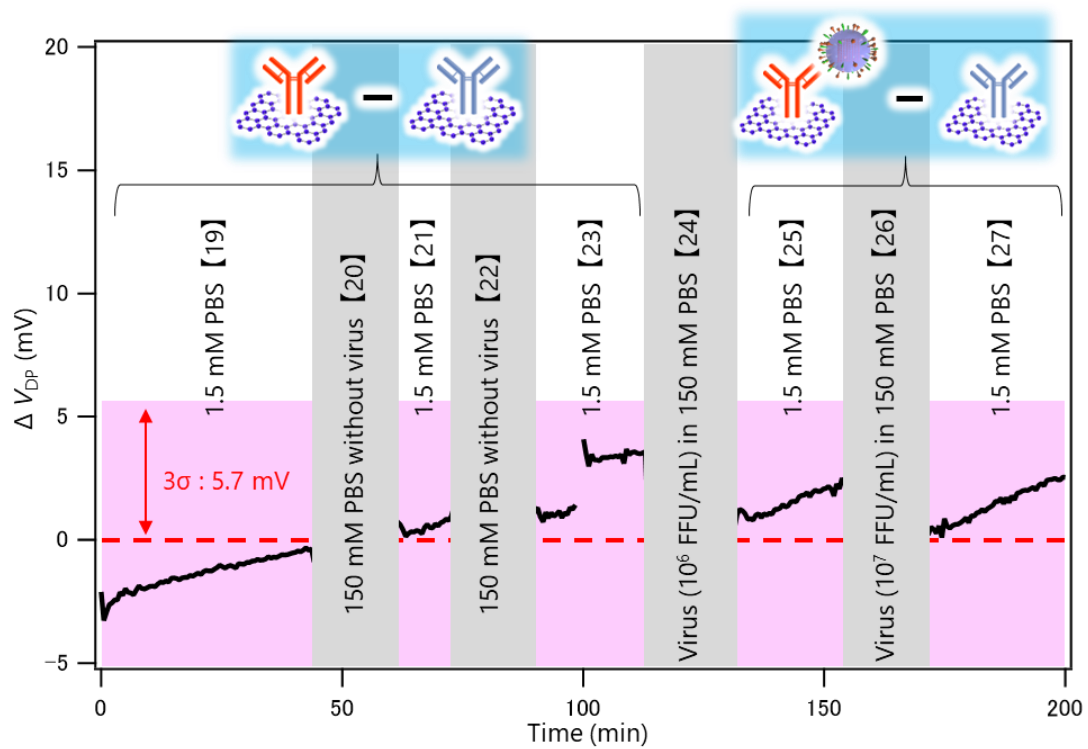


Fig. 5.6. Result of detection of SARS-CoV-2 using graphene field-effect transistor (GFET) modified by SARS-CoV-2 spike antibody (Ab) and by influenza H10N8 Hemagglutinin Ab. Both FETs are NOT modified with poly-L-lysine. Graph is calculated result by subtracting mean of Dirac point of GFET modified by influenza H10N8 Hemagglutinin Ab from that by SARS-CoV-2 spike Ab.

5.3.5 Surface observation using SEM

Viruses on GFET were observed by SEM. Figs. 5.7(a), (b), (c) and (d) shows the SEM image of probe FET with PLL (area (i) in Fig. 5.2(c)), that without PLL (area (ii) in Fig. 5.2(c)), reference FET with PLL (area (iii) in Fig. 5.2(c)) and that without PLL (area (iv) in Fig. 5.2(c)). Viruses bind well with Ab as shown in Figs. 5.7(a) and (b) because virus density is higher than Fig. 5.7(c) and (d). The ΔV_{DP} shifted by +10 mV as shown in Fig. 5.5 because of the difference of the number of virus particles shown in Figs. 5.7(a) and (c). On the other hand, the ΔV_{DP} was not shifted as shown in Fig. 5.6 despite the difference in the number of virus particles shown in Figs. 5.7(b) and (d). Despite a comparable difference in the number of virus particles, the magnitude of the ΔV_{DP} was different. This effect is caused by the presence or absence of PLL. The blocking effect of Tween 20 was confirmed because the number of viruses in Figs. 5.7(c) and (d) used as reference FETs is significantly smaller than that in Figs. 5.7(a) and (b) used as probe FETs.

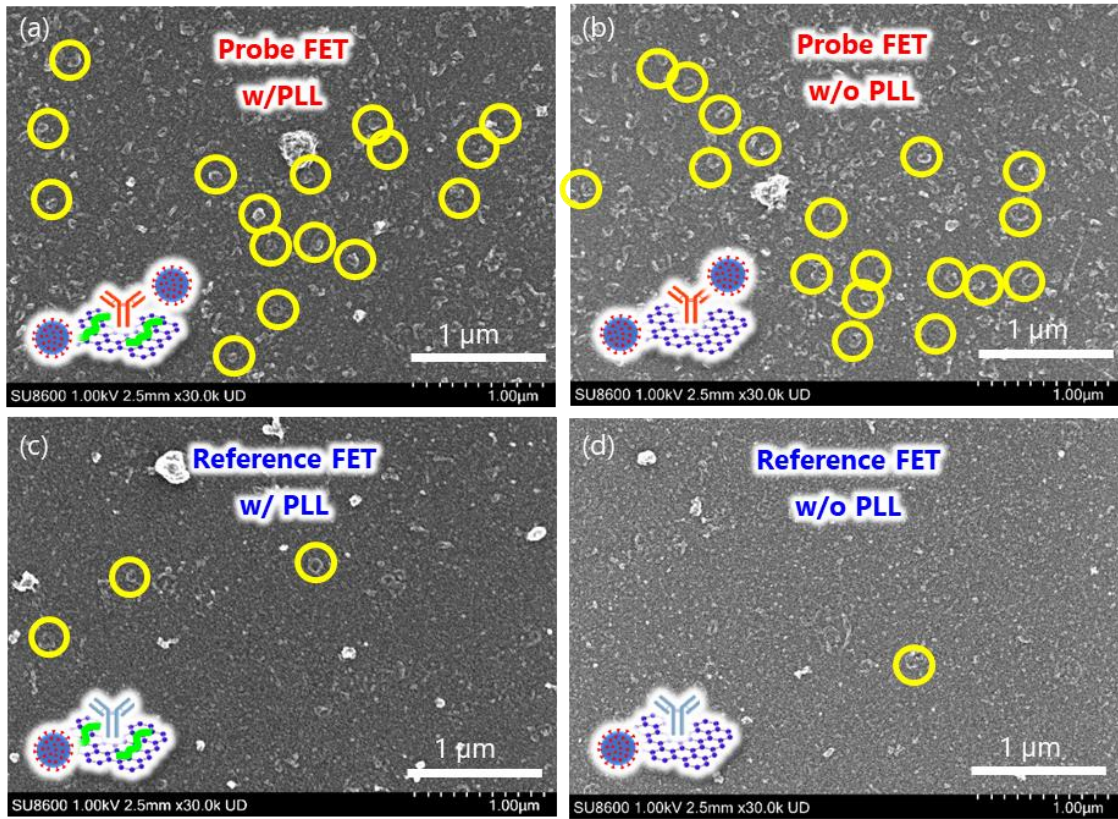


Fig. 5.7. SEM images of each area. (a) Probe field-effect transistor (FET) with poly-L-lysine PLL). (b) Probe FET without PLL. (c) Reference FET with PLL. (d) Reference FET without PLL.

5.4 Detection Results of SARS-CoV-2 using acetate buffer

5.4.1 Introduction of detection results of SARS-CoV-2 using acetate buffer

To date, we have reported detection of SARS-CoV-2 using a GFET modified with PLL in 1.5 mM PBS solution⁹. In this study, we sought to improve sensitivity by replacing 1.5 mM PBS (pH 7) with 1 mM acetate buffer (pH 4), since its pH is farther from the SARS-CoV-2 isoelectric point (pI 5.2–6.1) than that of PBS^{10,11}. Although the spike proteins of SARS-CoV-2 carry positive charges¹², the viral lipid bilayer and RNA bear numerous negative charges in PBS; this charge distribution underlies the virus' pI. We have addressed major GFET challenges, such as baseline drift, charge effects from non-specifically adsorbed virus, and noise, by subtracting the shift in transfer characteristics of reference FET from that of probe FET^{13,14}. We also estimated the sensitivity dependence on graphene surface charge density in acetate buffer by solving the Poisson–Boltzmann equation.

5.4.2 Detection of SARS-CoV-2 using GFET without PLL in acetate buffer

A 1 mM acetate buffer at pH 4 was used for SARS-CoV-2 detection to compare sensitivity with that of PBS buffer at pH 7. To determine V_{DP} , I_{SD} was measured at each point by sweeping the gate voltage from -100 to 400 mV. V_{DP} was then calculated as the V_{GS} value at which I_{SD} reached its minimum. Fig. 5.8 shows the calculated result of ΔV_{DP} values plotted over time. Fig. 5.8 presents the result of SARS-CoV-2 detection using GFET without PLL. The horizontal and vertical axes represent the time and the ΔV_{DP} , respectively. ΔV_{DP} was calculated by subtracting the mean V_{DP} of the reference FETs with Influenza Ab (area (iv) in Fig. 5.2(c)) from that of the probe FETs with SARS-CoV-2 Ab (area (ii) in Fig. 5.2(c)) and is shown as the solid black line. Although signal drifts were not completely eliminated ([A], [C], and [E]), the drifts of ΔV_{DP} became relatively smaller. Prior to viral addition (F), 150 mM PBS was added twice ([B] and [D]) to assess the effect of disturbances on ΔV_{DP} due to buffer solution exchanges and to estimate the noise floor of the system. The dotted line indicates the mean ΔV_{DP} of the acetate buffer before the viral addition ([A], [C], and [E]), while the shaded area indicates the noise floor, calculated as three times the standard deviation ($3\sigma = 3.1$ mV). Tween 20 (0.01 %) was added to all solutions during these measurements. Buffer exchanges from 150 mM PBS to 1 mM acetate buffer were carried out to extend the Debye length. Buffer exchanges were performed only when the V_{DP} fluctuation in the acetate buffer was less than < 0.2 mV/min. Notably, after the addition of SARS-CoV-2 at a concentration of 1×10^7 FFU/mL (F), ΔV_{DP} shifted by approximately -10 mV, even without PLL (G). This negative shift surpassed the noise floor, indicating successful virus detection by the measurement system. The negative shift of ΔV_{DP} is reasonable, as SARS-CoV-2 virus is expected to be positively charged in the acetate buffer at pH 4, given its pI value of 5.2–6. Moreover, it can be said that the viral charge in acetate buffer is detected using GFET without PLL.

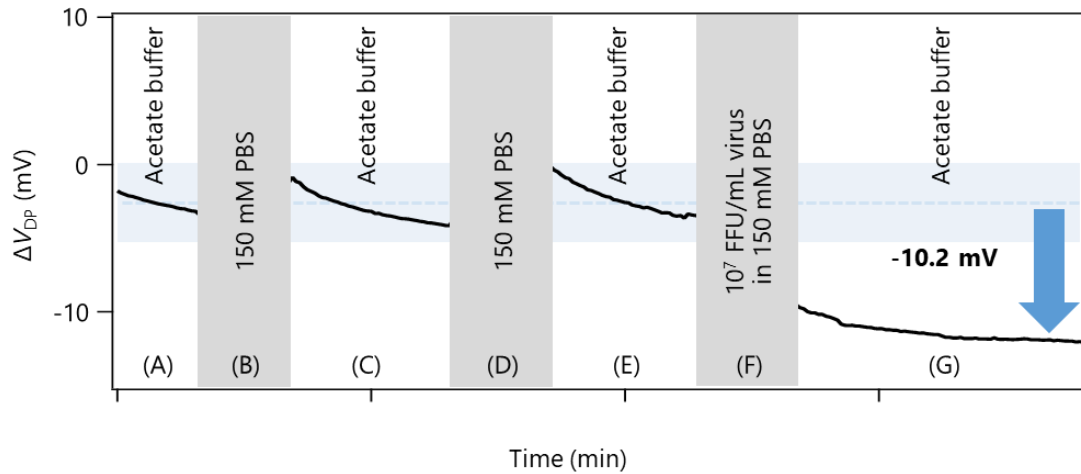


Fig. 5.8. Virus measurement results in acetate buffer using graphene field-effect transistor (GFET). The solid line indicates the calculated difference between the mean of the probe FETs with SARS-CoV-2 antibodies (Abs) and the reference FETs with influenza Abs. Poly-L-lysine was not modified.

5.4.3 Detection of SARS-CoV-2 using GFET with PLL in acetate buffer

Simultaneously, virus detection using GFET with PLL was performed on the same substrate. Fig. 5.9 illustrates the results of these measurements using GFET with PLL in 1 mM acetate buffer at pH 4. The horizontal and vertical axes represent the time and the ΔV_{DP} , respectively. ΔV_{DP} was calculated by subtracting the mean V_{DP} of the reference FETs with Influenza Ab and PLL (area (iii) in Fig. 5.2(c)) from that of the probe FETs with SARS-CoV-2 Ab and PLL (area (i) in Fig. 5.2(c)), and represents by the solid black line. The noise floor was calculated as three times the standard deviation of ΔV_{DP} of (A), (C), and (E), and $3\sigma = 2.7$ mV. Tween 20 (0.01 %) was included in all solutions during this measurement. Buffer exchanges were performed when the fluctuation in V_{DP} in acetate buffer was less than 0.2 mV/min. Following the addition of acetate buffer (E), SARS-CoV-2 at a concentration of 1×10^7 FFU/mL (F) was introduced and then exchanged with fresh acetate buffer (G) to assess the ΔV_{DP} shift. Unfortunately, after adding SARS-CoV-2 at 1×10^7 FFU/mL (F), only a small ΔV_{DP} shift of -5.9 mV (G) in the negative direction was observed even with PLL, which surpassed the noise floor, confirming virus detection in the measurement system. Although the surface potential density was modulated using PLL with positive charge, the obtained signal was smaller (-5.9 mV) compared to that without PLL (-10 mV). This discrepancy compared to the case of PBS buffer is discussed further in the following Section 5.4.5

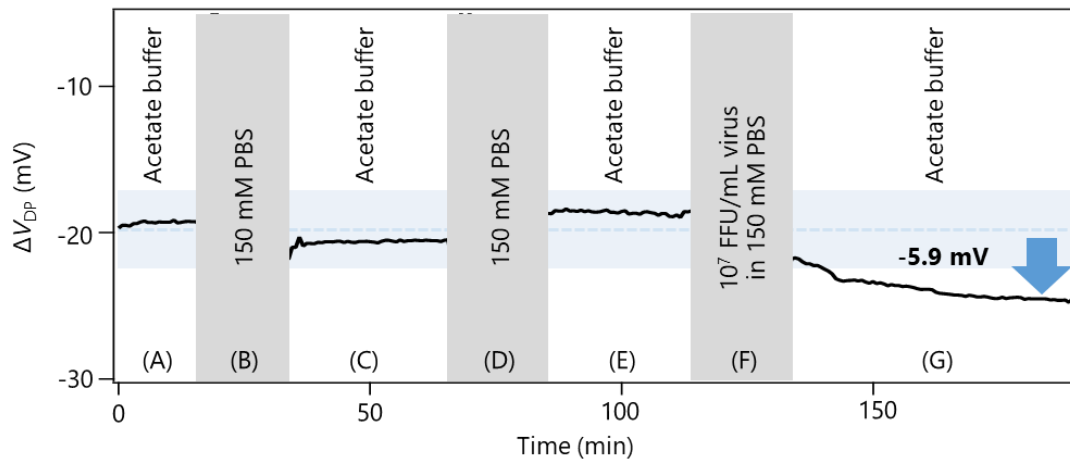


Fig.5.9. Virus measurement results in acetate buffer using a graphene field-effect transistor (GFET). The solid line indicates the calculated difference between the mean of the probe FETs with SARS-CoV-2 antibodies (Abs) and the reference FETs with influenza Abs. Poly-L-lysine was modified.

5.4.4 Measurement of charge of SARS-CoV-2 antibody in acetate buffer

To elucidate the discrepancy observed in Fig. 5.9, the charges of SARS-CoV-2 Abs in acetate buffer were measured. Abs in this buffer are expected to be positively charged. To evaluate this, a total of 32 GFETs were divided into two regions. In one region, SARS-CoV-2 Ab in 150 mM PBS was introduced onto half of the GFET area using same the procedure described in Section 5.2.1. In the other half of the GFET area, only 150 mM PBS was introduced onto graphene as a negative control, following the modification with PBASE, to compare against the half of the GFET area modified with Ab.

Transfer characteristics were measured by sweeping the V_{GS} ranging from 0 to 500 mV, while maintaining a source-drain voltage at 100 mV. Fig. 5.10(a) represents the means of the transfer characteristics of GFET before and after Ab modification, and Fig. 5.10(b) represents the means of the transfer characteristics of GFET before and after 150 mM PBS introduction, respectively. The horizontal and vertical axes represent the V_{GS} and I_{SD} in both figures. In Fig. 5.10(a), the dotted and solid lines indicate the GFET's transfer characteristics before and after the Ab modification, respectively. On the other hand, in Fig. 5.10(b), the dotted and solid lines indicate the GFET's transfer characteristics before and after the introduction of 150 mM PBS, respectively. All measurements were performed in 1 mM acetate buffer. The transfer characteristic shifted in the negative direction as shown in Fig. 5.10(a), on the other hand, that shifted in the positive direction as shown in Fig. 5.10(b). As the DPs exceeded the measurement limit ($V_g = 500$ mV) in our system, shown in Figs. 5.10(a) and (b), we could not precisely evaluate the exact DP shift. Instead, we compared the difference in drain currents (ΔI_{SD}) at a constant gate voltage of 300 mV before and after Ab modification. Fig. 5.10(c) represents the deference of ΔI_{SD} for Fig. 5.10(a) and (b). The vertical axis represents ΔI_{SD} . The red line depicts the mean and standard deviation for GFETs with SARS-CoV-2 Ab modification, and the blue line depicts that without Ab modification, serving as a negative control. The mean ΔI_{SD} with Ab modification is $-88.2 \mu A$, with a standard deviation of $48.3 \mu A$. Conversely, the mean ΔI_{SD} without Ab modification is $+8.0 \mu A$, with a standard deviation of $60.5 \mu A$. Comparing these shifts in I_{SD} before and after Ab modification demonstrates that electrons were induced in graphene due to positive charges of Ab. This finding supports the measurement results in Figs. 5.8 and 5.9 as follows: As previously stated, the SARS-CoV-2 Ab was positively charged in 1 mM acetate buffer. These positive charges within the Debye length effectively neutralize the net surface charge density of graphene. This compensation enhances the sensitivity of GFET even without PLL modification. In contrast, when PLL is added to GFET, the net surface charge density of the GFET exceeds the highest sensitivity point of $Q_s/eS = 0$ because PLL become more positively charged¹⁵. Consequently, the sensitivity decreases compared to GFETs without PLL modification.

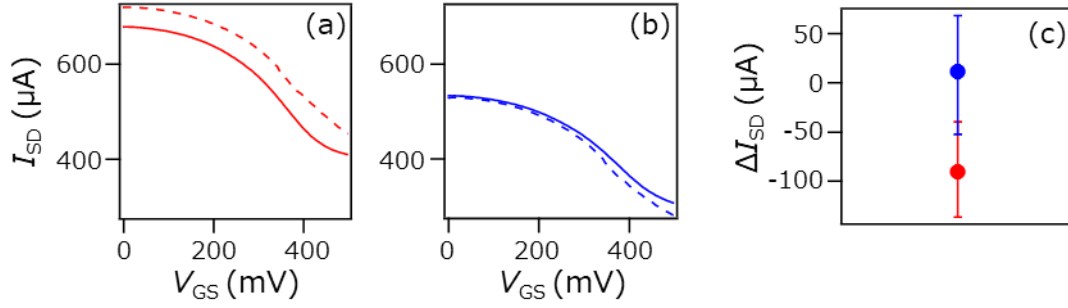


Fig. 5.10. (a) Transfer characteristics of graphene field-effect transistor (GFET) before and after SARS-CoV-2 antibody (Ab) modification using 1 mM acetate buffer. The dotted and solid lines indicate GFET's transfer characteristics before and after Ab modification, respectively. (b) Transfer characteristics of GFET without Ab modification. The dotted and solid lines indicate GFET's transfer characteristics before and after the introduction of 150 mM PBS, respectively. (c) The difference in ΔI_{DS} at a constant gate voltage of 300 mV in 1 mM acetate buffer. The red line depicts mean and standard deviation for GFETs with SARS-CoV-2 Ab modification, and the blue line represents GFETs without Ab modification, serving as a negative control.

5.4.5 Comparison of the sensitivity in PBS and acetate buffer

Here, the sensitivity of GFET in PBS and acetate buffer was compared and the detected result is shown in Fig. 5.11(a) and (b). Fig. 5.11 is based on Fig. 4.1 with the addition of the result of four detection. Fig. 5.11(a) shows the results of viral detection using GFET with and without PLL in PBS. The viral charge was not detected using GFET without PLL. However, by adding PLL, viral charge was detected because ΔV_{DP} shifted + 10.0 mV. These results suggest that the sensitivity was increased because positive charge of PLL shifted the surface charge density near to zero. On the other hand, Fig. 5.11(b) represents the results of virus detection using GFET with and without PLL in acetate buffer. Although the viral charge was detected with or without PLL, the ΔV_{DP} of GFET without PLL was larger than that with PLL. These results suggest that the sensitivity was decreased because PLL charged more positively in acetate buffer, and the sensitivity exceeded the highest point of zero surface charge density.

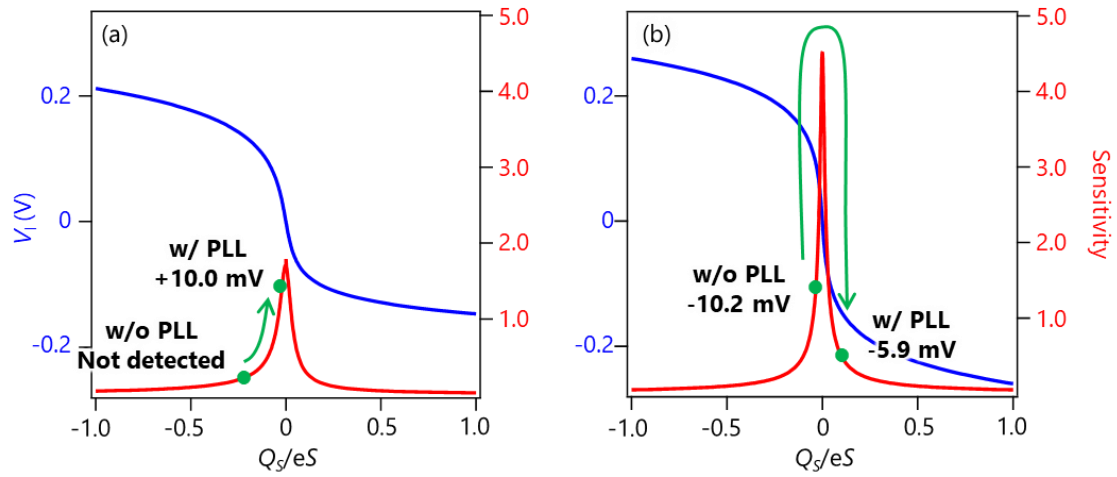


Fig. 5.11. Interpretation of sensitivity of graphene field-effect transistor in (a) PBS and (b) acetate buffer with and without poly-L-lysine introduction.

5.4.6 Surface observation using SEM

Viruses on GFET were observed by SEM. Fig 5.12 (a) and (b) shows the SEM image of probe (area (ii) in Fig. 5.2(c)) and reference (area (iv) in Fig. 5.2(c)) FET without PLL, respectively. Viruses bind well with Ab as shown in Fig. 5.12(a) because virus density is higher than Fig. 5.12(b). Moreover, the blocking effect of Tween 20 was confirmed.

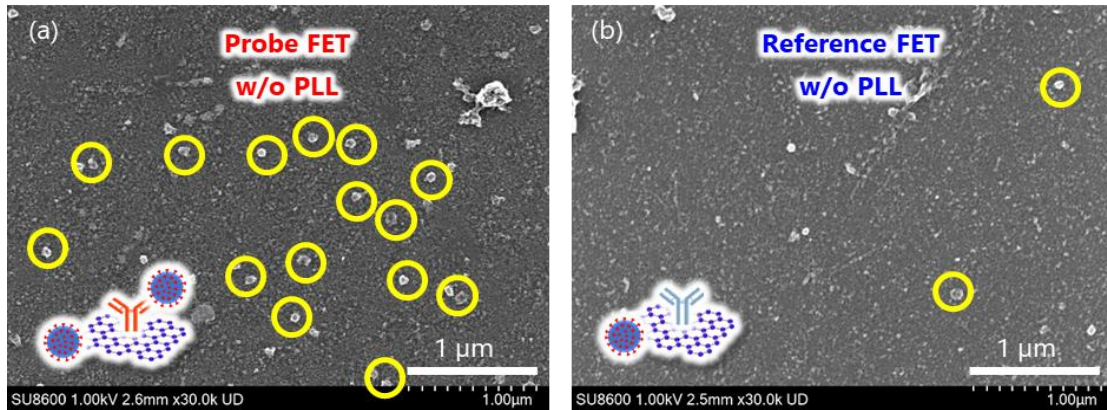


Fig. 5.12. Scanning electron microscope images of (a) probe field-effect transistor (FET) without poly-L-lysine (PLL) and (b) reference FET without PLL.

5.5 Summary

Using GFET modified with SARS-CoV-2 spike Ab and PLL used as the ζ potential modulator, SARS-CoV-2 in PBS was detected. By applying PLL on GFET biosensor, we have succeeded in the great enhancement of the sensitivity of GFET. The effect of the physical adsorption of the viral charge and that of the drift could be removed by using the reference FETs modified with influenza H10N8 Hemagglutinin Ab. Also, the charge detection of SARS-CoV-2 was successfully demonstrated in acetate buffer using GFET both with and without PLL modulation and compared with results from the previous study using PBS. The charge of the virus becomes negative in PBS at pH 7. Conversely, it becomes positive in the acetate buffer at pH 4, as the pI of SARS-CoV-2 ranges from 5.2 to 6.1. The sensitivity of GFET was increased in PBS buffer when PLL was modified on GFETs (+10 mV). However, it decreased in the acetate buffer when PLL was modified on GFETs (-5.9 mV) due to the positive charge of PLL.

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6 Stabilization of the Dirac point by controlling leakage current and detection of virus

6.1 Introduction of stabilization of Dirac point by leakage current control

Our measurement method suffered from the drift problems. Previously, we used a method of subtracting the mean of V_{DP} of reference FETs from that of probe FETs¹ to remove the effect of drift characteristics. In this study, we identified one of the causes of this drift is due to leakage current between a reference electrode and drain metal electrodes. The metal electrodes were insulated by passivation layer for the leakage current, and drastically reducing drift during virus detection was achieved. As a result, the waiting time for drift stabilization was eliminated, enabling both rapid and highly sensitive measurements.

6.2 Drift characteristics of gate leakage current with and without passivation layer

6.2.1 Drift characteristics of gate leakage current without passivation layer

The gate leakage current was analyzed in detail. Leakage current measurements were performed under the following conditions: V_{GS} was applied to the drain electrode through PBS using an Ag/AgCl electrode as shown in Fig. 6.1(a). 150 mM PBS and the microchannel were used for these measurements. During the V_{GS} sweep, leakage current was measured using custom-built equipment.

The gate leakage current of the 32 GFETs without passivation layer was measured as a function of the V_{GS} as shown in Fig. 6.1(b). The horizontal axis is the V_{GS} , and the vertical axis is the gate leakage current. The 32 graphs correspond to 32 GFETs. The gate leakage currents can be classified by two categories, i.e., the high gate leakage current, and the low gate leakage current. These differences may come from the area of the metal drain electrode. Therefore, the areas of the metal drain electrodes for the high and low gate leakage current were calculated. The high gate leakage current group corresponds to a large drain electrode area of 0.58 mm² and the low gate leakage current group corresponds to a small drain electrode area of 0.28 mm², respectively. A gate leakage current density was then calculated by dividing the gate leakage current by the area of the metal drain electrode at the gate voltage of 500 mV. As a result, the gate leakage current density of large-area drain electrode and the small-area drain electrode were almost similar values of 0.58 and 0.73 $\mu\text{A}/\text{mm}^2$, respectively. These results mean that the gate leakage current really occurs between the reference electrode and the metal drain electrode. Therefore, the difference of the high and low gate leakage currents in Fig. 6.1(b) came from only the difference of the area of the drain metal electrode in the solution.

The dependence of the gate leakage current at 500 mV on the time was measured and shown in Fig. 6.1(c). The horizontal axis is the time, and the vertical axis is the gate leakage current. The gate leakage current shows the drift characteristic and reduced drastically along the time. The reason for the drift of the gate leakage current is not clear so far. But the possible three reasons are 1) The surface of the

metal drain electrode was gradually passivated by the contaminant in the solution. 2) The reference electrode becomes degraded because of the relatively large gate leakage current. 3) Oxidation-reduction reaction occurs at the interface between the Ti/Au metal electrode and the electrolyte.

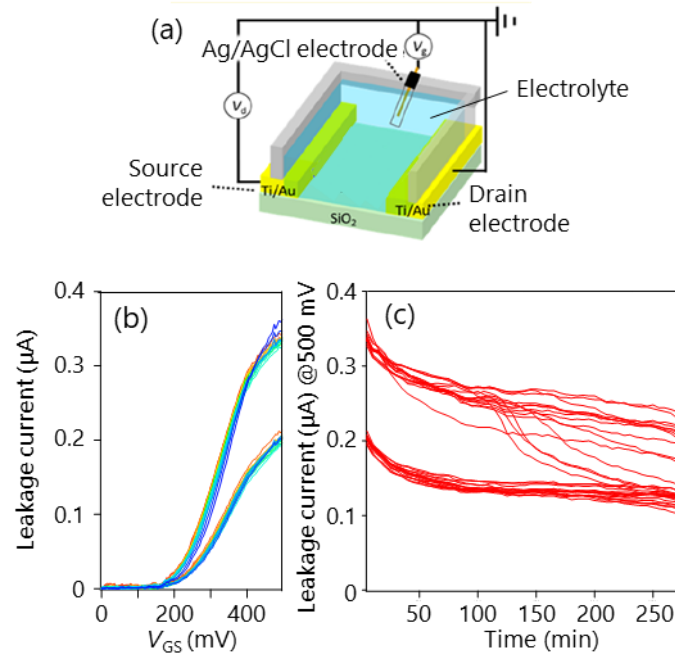


Fig. 6.1. (a) Illustration of environment for gate leakage current measurement. (b) Gate leakage current of 32 graphene field-effect transistors without passivation layer. (c) Dependence of gate leakage current on time at 500 mV.

6.2.2 Measurement of gate leakage current for various passivation layer

The gate leakage currents between the drain and the reference gate electrodes were measured in the 150 mM PBS solution without (a), with SU-8 (b), and SiO₂ (c) passivation layers, and shown in Fig. 6.2. The substrate without passivation layer shows the large gate leakage currents as shown in Fig. 6.2(a) at the positive gate bias of ~ 500 mV. The gate leakage currents insulated by SU-8 and measured in the same manner as in Fig. 6.2(a) are shown in Fig. 6.2(b). In this case, the gate leakage currents were reduced relatively. Moreover, the gate leakage currents insulated by SiO₂ and measured in the same manner as in Fig. 6.2(a) is shown in Fig. 6.2(c). In this case, the leakage currents were dramatically reduced and almost out of the range of measurement. This reduction of the gate leakage currents should be the main reason for the stable time measurement of the biosensing as will be described in Section 6.4.

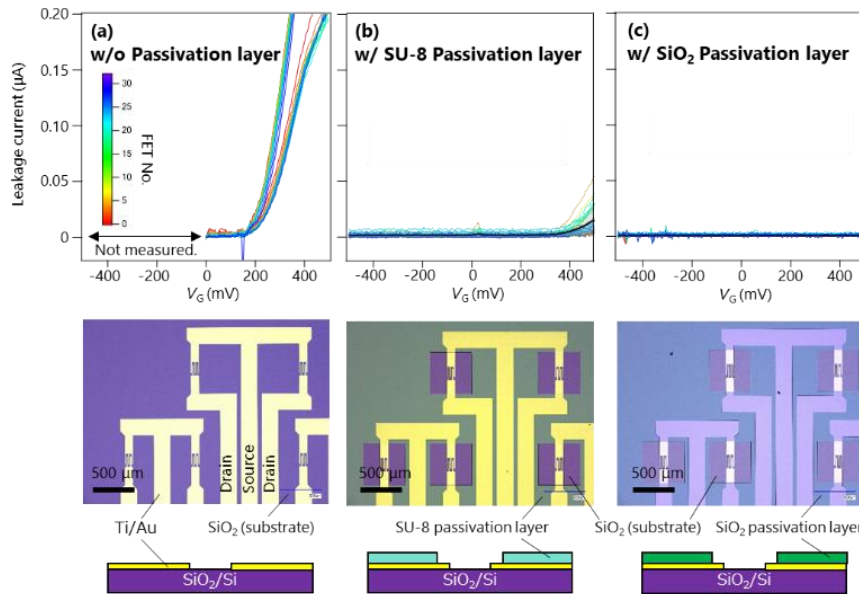


Fig. 6.2. Gate leakage currents between drain and reference gate electrodes measured in 150 mM PBS solution (a) without, with (b) SU-8 and (c) SiO₂ passivation layers. From top to bottom, the leakage current, optical images of silicone substrate, and its cross-sectional views.

6.3 Procedure of GFET with passivation layer on metal electrodes

To compare the V_{DP} drift, GFET with and without passivation layer were fabricated. The GFET arrays were fabricated as shown in Fig.6.3 and this fabrication procedure is the same as that described in Section 2.3. Source and drain electrodes were formed by depositing 10 nm/90 nm of Ti/Au on substrates. One substrate had no passivation layer shown in the upper part in Fig. 6.3, and the other substrate had passivation layer of SU-8 or SiO_2 , shown in the lower part in Fig. 6.3. The graphene film was then transferred using PMMA onto the prepared substrates, and patterned via oxygen plasma etching, resulting in the integration of 32 GFETs on these substrates. The PMMA film was dissolved in ethanol. Optical images of a typical GFET with and without passivation layer are shown in the right side of Fig. 6.3. In the optical image with passivation layer, only the channel part is opened. The channel length and width are 10 and 400 μm , respectively.

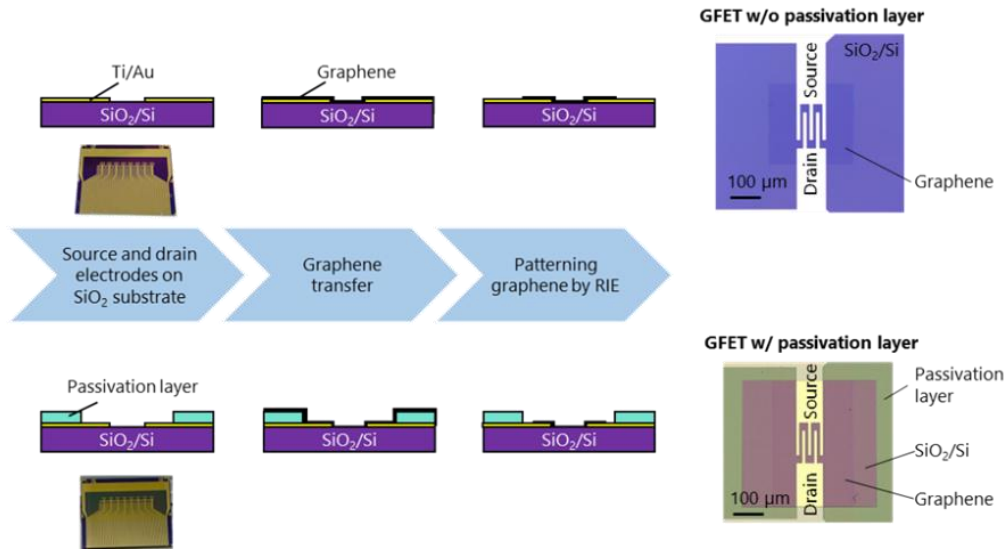


Fig. 6.3. Fabrication process of graphene field-effect transistor (upper part) without and (lower part) with passivation layer.

6.4 Comparison of drift between GFET with and without passivation layer

In the verification of V_{DP} drift, the silicone substrate with Ti/Au electrodes with and without the passivation layer were prepared and GFET was fabricated as shown in Fig.6.3. The V_{GS} was applied through the buffer solution using an Ag/AgCl electrode. During the V_{GS} sweep, I_{SD} was measured using custom-built equipment.

The drift characteristics of the V_{DP} of GFET with (the red line in Fig. 6.4) and without (the blue line in Fig. 6.4) SU-8 passivation layer was compared. The horizontal axis is the time, and the vertical axis is the V_{DP} . The red and blue graphs represent the means of the V_{DP} of 24 GFET with SU-8 passivation layer and that without SU-8, respectively. The shaded area represents the standard deviation. For the measurement of the virus charge as shown in section 3.3.3, two concentrations PBS were used, i.e., 1.5mM PBS to enhance the sensitivity for the longer Debye length, and 150 mM PBS for the stronger connection of virus to the receptor²³⁴⁵. In addition, it also serves to evaluate the disturbance caused by the introduction of 150 mM PBS. Thus, 1.5 mM PBS was exchanged two times to 150 mM PBS without virus. The microchannel was used to flow these electrolytes. Without passivation layer, as the blue line shows, the V_{DP} drifts are as large as 0.36 mV/min in the 1.5 mM PBS. On the other hand, as the red line shows, the drift of the V_{DP} is drastically reduced and becomes as small as 0.06 mV/min. As a result, the SU-8 passivation layer enables to reduce the drift drastically. It turns out that one of the origins of the drift characteristics is the gate leakage current between the reference electrode and the drain electrode in the buffer solution. Therefore, a stable bio detection by GFET can be expected by suppressing the gate leakage current.

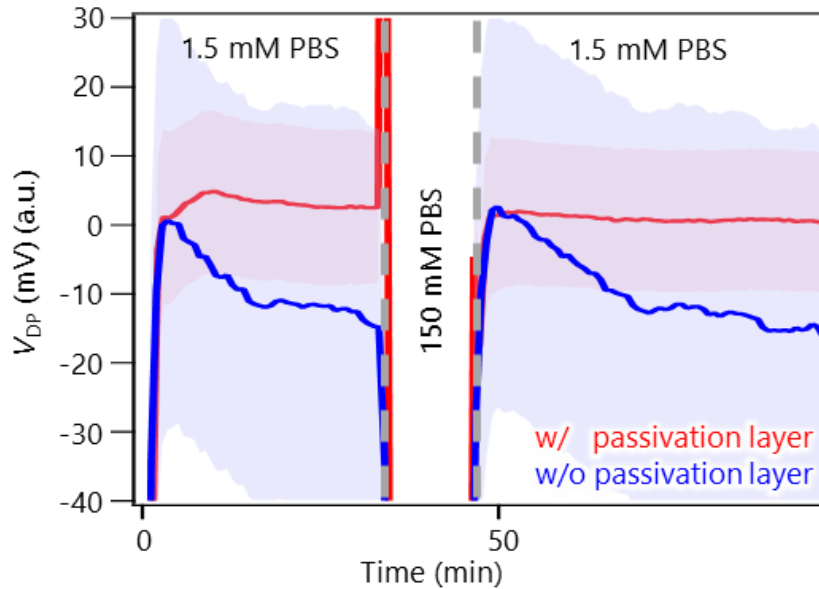


Fig. 6.4. Drift characteristics of V_{DP} of graphene field-effect transistor with (the red line) and without (the blue line) passivation layer.

6.5 Biosensing application using glycan functionalized GFET with passivation layer

6.5.1 Introduction of glycan functionalized GFET

In the near future, it is said that the novel influenza virus may spread rapidly same as SARS-CoV-2. Avian influenza virus often infects the swine and it sometimes changes its structure and may get a human infection. Therefore, the pandemic will occur due to this novel human infectious influenza virus. Human influenza viruses combined with Sia α 2,6-Gal^{6,7} while avian influenza virus combine with Sia α 2,3-Gal⁸. Sia α 2,6-Gal are present on epithelial cells in the human trachea while Sia α 2,3-Gal is deep on alveolar cells. This mechanism was applied to a GFET-based platform to assess the human infectivity of novel infectious influenza viruses. In this study, glycan-functionalized GFET was fabricated and avian H9N2 influenza virus was detected. The transfer characteristics of GFET shift because the influenza virus carry negative charges in PBS.

6.5.2 Purpose

This study aims to achieve high sensitivity detection of avian H9N2 influenza viruses using glycan functionalized GFET. This substrate was insulated by SiO₂ passivation layer as described in Section 6.2.2. Moreover, we aimed to improve the sensitivity of GFET using glycan. Because glycan is smaller than Ab, virus binds closer to the graphene, leading to larger V_{DP} shift.

6.5.3 Sialylglycopeptide

The structure and function of sialylglycopeptide (SGP) is described below. Fig. 6.5(a), (b), and (c) represents $\alpha 2,6$ -SGP, $\alpha 2,3$ -SGP, asialo-SGP, respectively. SGP consists of glycan and peptide as shown in Fig. 6.5. $\alpha 2,6$ -SGP binds to human-infective influenza virus because it includes Sia $\alpha 2,6$ -Gal structure. On the other hand, $\alpha 2,3$ -SGP binds to avian-infective influenza virus, as it includes Sia $\alpha 2,3$ -Gal structure. These SGPs have terminal sialic acid residue, followed by glucose. Also, asialo-SGP do not have sialic acid and is used generally for reference of $\alpha 2,6$ -SGP and $\alpha 2,3$ -SGP.

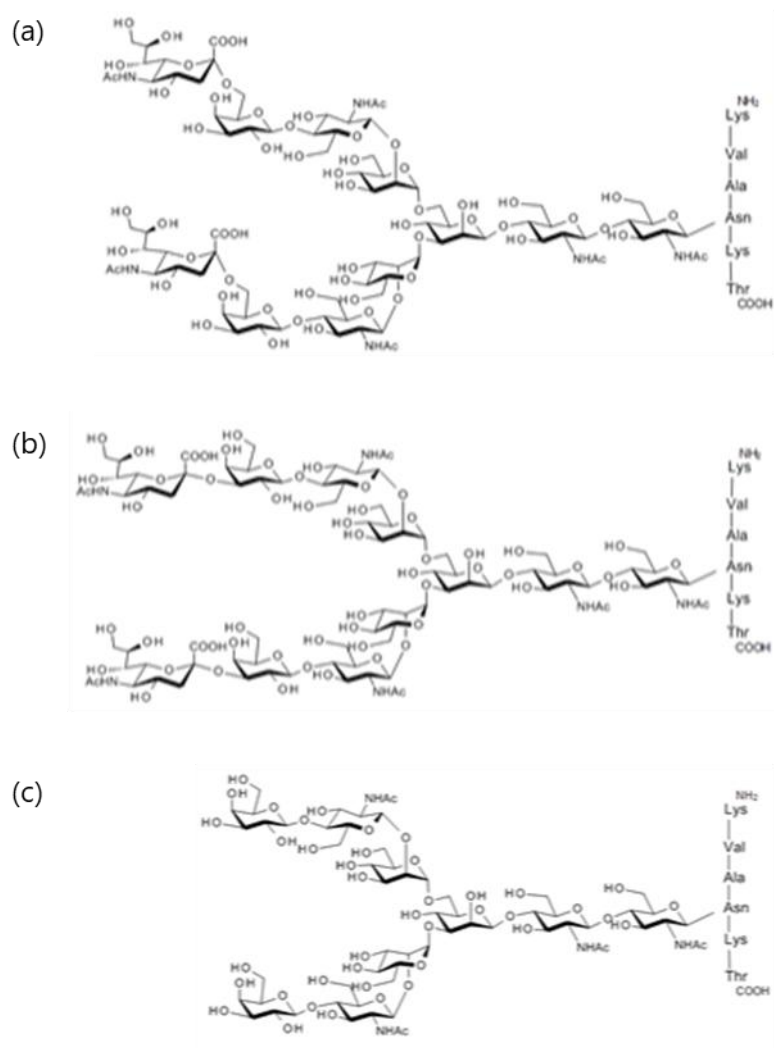


Fig. 6.5. Structures of (a) $\alpha 2,6$ -Sialylglycopeptide (SGP), (b) $\alpha 2,3$ -SGP, and (c) asialo-SGP.

6.5.4 Procedure of glycan functionalized GFET and confirmation of pyrene-SGP modification

The channel length and width of GFET are 10 and 400 μm , respectively. As in Section 3.3.1, we tried to modify the-SGP on graphene surface instead of antibodies. However, PBASE and SGP did not bind, probably due to the negative charge of SGP in PBS. Therefore, PBASE and SGP bind in advance and named pyrene-SGP. This pyrene-SGP was isolated by extraction and HPLC. 1 mM pyrene-SGP was dissolved in pure water and introduced on GFET. After the modification for two nights at 40 $^{\circ}\text{C}$, pyrene-SGP solution was removed and GFET was washed by 1.5 mM PBS.

The modification was evaluated by transfer characteristics shift. Fig. 6.6(a) represents the results of pyrene- $\alpha 2,3$ -SGP modification. Fig. 6.6(b) represents the reference results. These measurements were performed using 1.5 mM PBS. The horizontal and vertical axes are the V_{GS} and the I_{SD} , respectively. In Fig. 6.6(a), the dotted and solid lines are the transfer characteristics before and after pyrene-SGP modification. In Fig. 6.6(b), the dotted and solid lines are the transfer characteristics before and after the introduction of pure water. In Fig 6.6(a), transfer characteristic shifts in the positive direction because the π electrons of pyrene induce holes in graphene. Conversely, transfer characteristics hardly shifted in Fig. 6.6(b). Based on these results, pyrene-SGP was considered to be modified on graphene.

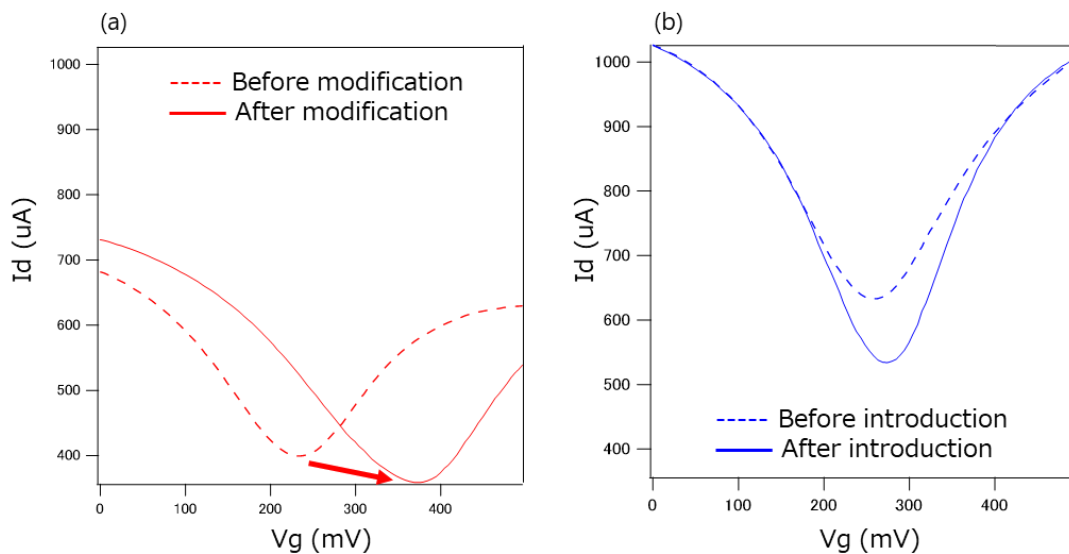


Fig. 6.6. Transfer characteristics of graphene field-effect transistor before and after (a) modification of pyrene-SGP and (b) introduction of its solvent.

6.5.5 Detection results of avian H9N2 influenza virus using GFET with passivation layer

Fig. 6.7(a) represents the results of the detection of avian H9N2 influenza virus by GFET with SiO₂ passivation layer. The horizontal axis is the time and the vertical axis is the DP of GFET. The red, green, and light blue solid lines represent the DP of GFET modified with pyrene- α 2,6-SGP, pyrene- α 2,3-SGP, pyrene-asialo-SGP, respectively, and the blue solid line represents that of unmodified GFET. GFET modified with pyrene- α 2,3-SGP is used for the probe FET and the others for the reference FETs. These DPs were highly stable because the SiO₂ passivation layer reduced the leakage current as proved previously. Buffer exchanges of 150 mM PBS to 1.5 mM PBS were performed to extend the Debye length. Before viral addition (F), 150 mM PBS ([B] and [D]) were added to assess the effect of the disturbances on DP owing to the buffer solution exchanges and to calculate the noise floor of the system. After adding the avian H9N2 influenza virus at concentrations of 4, 40, and 400 HAU/50 μ L ([F], [H], and [J]), 1.5 mM PBS ([G], [I], and [K]) were replaced on GFET.

By subtracting the mean value of the DP of the reference GFETs modified with 2,6-SGP from that of the probe GFET modified with 2,3-SGP, ΔV_{DP} was calculated and shown in Fig. 6.7(b). The dotted line in Fig. 6.7(b) indicates the mean value of ΔV_{DP} at 1.5 mM PBS before the viral addition ([A], [C] and [E]), and the shaded area indicates the noise floor (3σ) calculated as three times of the standard deviations. The effects of the drift and the charge of physically adsorbed virus could be reduced by this calculation. After the introduction of avian H9N2 influenza virus at concentrations of 4 and 40 HAU/50 μ L ([G], [I]), V_{DP} did not exceeded 3σ . After the introduction of avian H9N2 influenza virus at a concentration of 400 HAU/50 μ L (J), ΔV_{DP} moved up to approximately +7.5 mV (K) and exceeded 3σ . This positive shift is attributed to the negative charges of avian H9N2 influenza virus in PBS. Thus, the infection of avian H9N2 influenza virus was successfully detected using glycan-functionalized GFET.

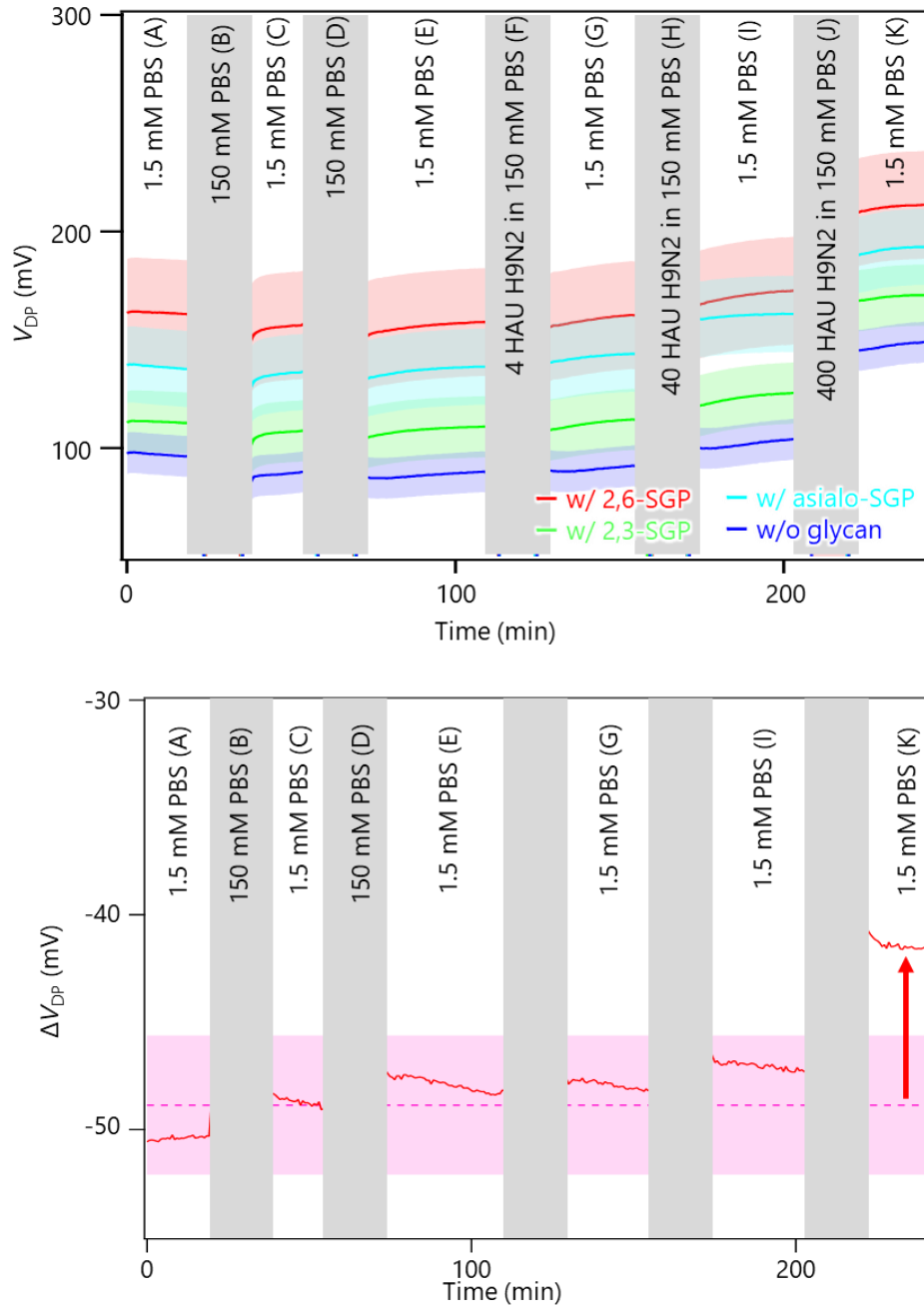


Fig. 6.7. (a) Detection of avian H9N2 influenza virus by graphene field-effect transistor (GFET). Red, green, and light blue solid lines represent Dirac point of GFET modified with 2,6-SGP, 2,3-SGP, asialo-SGP, respectively. Blue solid line represents that with unmodified GFET. Shaded areas are standard deviations. GFET modified with 2,3-SGP is used as a prove FET and the others are for the reference FET. (b) Dependence of ΔV_{DP} on time calculated by subtracting mean value of V_{DP} of reference GFETs with 2,6-SGP from that of probe GFETs with 2,3-SGP. After the introduction of avian H9N2 influenza virus at a concentration of 400 HAU/50 μ L, ΔV_{DP} shifted 7.5 mV.

6.6 Biosensing application using antibody functionalized GFET with passivation layer

6.6.1 Procedure of virus detection

Viral measurements were performed under the following conditions: V_{SD} was set to 100 mV. V_{GS} was swept from 0 to 500 mV in PBS. During the V_{GS} sweep, I_{DS} was measured for each GFET using custom-built equipment.

6.6.2 Detection results of SARS-CoV-2 using GFET with passivation layer

Using GFET with SiO_2 passivation layer, SARS-CoV-2 was detected. In this measurement, two microchannel areas shown in Fig. 6.8(a) were applied, and one area with 12 GFETs is used for the probe devices, and the other area with 14FETs for reference devices. All GFETs were modified with SARS-CoV-2 Ab. An Ab modification procedure to all GFETs for both areas follows previously reported methods^{9,10}. Inactivated virus (SARS-CoV-2) was prepared as an antigen.

In Fig. 6.8(b), the horizontal axis is the time, and the vertical axis is the V_{DP} . The red and blue graphs represent the means of the V_{DP} of probe GFETs and that of reference GFETs, respectively. The shaded area represents the standard deviation. After the introduction of 1.5 mM PBS and 150 mM PBS, SARS-CoV-2 at a concentration of 1×10^8 FFU/mL in 150 mM PBS was introduced into the probe FET area, and only 150 mM PBS without virus was introduced into the reference FET area. Then, the solutions were exchanged to 1.5 mM PBS for both areas to extend the Debye length for the measurement of V_{DP} . Throughout the measurement shown in Fig. 6.8(b), the reference GFETs shown by blue line shows no drift characteristics, though there are some fluctuations. Also, the probe FETs show the no drift characteristics just before the introduction of SARS-CoV-2. These no drift characteristics are attributed to the passivation layer to the metal source/drain electrodes which prevent the large gate leak current.

After the introduction of virus at a concentration of 1×10^8 FFU/mL, the V_{DP} of GFET drawn in red graph increased approximately 30 mV. This shift direction is correct because SARS-CoV-2 carry negative charges in PBS^{11,12} and V_{DP} shifted in the positive direction because the negative charges of virus induced holes in graphene¹³⁻¹⁶. On the other hand, the V_{DP} of the reference GFET introduced only 150 mM PBS shown in blue graph remained almost unchanged.

These results clearly show GFET with the passivation layer enable detection of the charge of virus with high sensitivity without drift problem. Moreover, correct V_{DP} value was obtained rapidly because V_{DP} drift was eliminated.

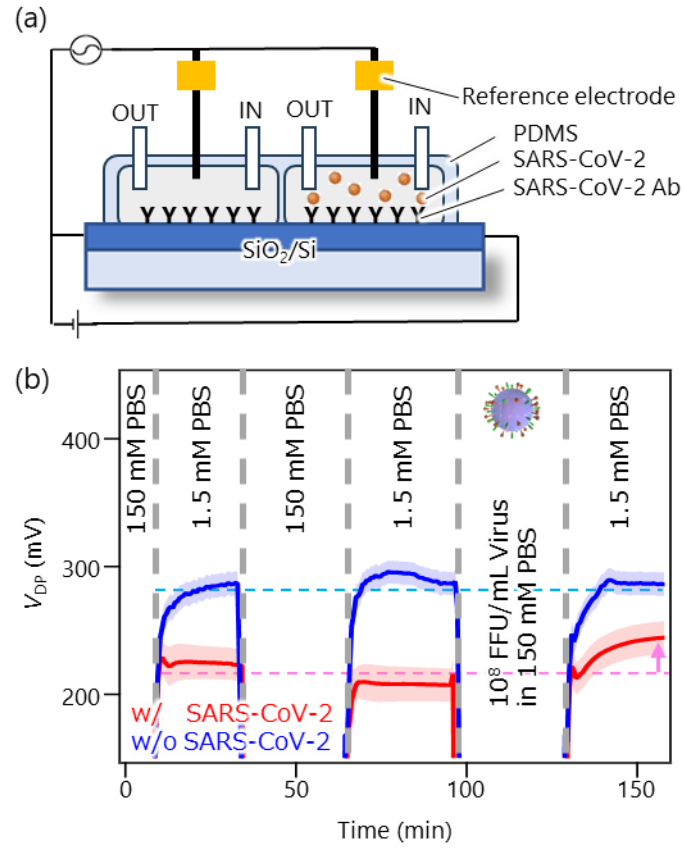


Fig. 6.8. (a) Illustration of environment for viral charge measurement using two micro-channel systems, one for probe field-effect transistors (FET), and the other for reference FET. (b) Virus measurement using graphene FET with passivation layer. The blue line indicates the V_{DP} of reference FETs, and the red line for probe FETs.

6.7 Summary

The major cause of the drift characteristics in the GFET biosensor was found to be the gate leakage current between the reference electrode and the metal source/drain electrode exposed to the solution. By insulating the metal source/drain electrode by SU-8 and/or SiO_2 layer, the drift characteristics were significantly improved by dramatically reducing this gate leakage current. When this method was used to detect avian H9N2 influenza virus and SARS-CoV-2, the drift characteristics were completely suppressed and a clear signal could be detected.

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7 Conclusions

Using GFET biosensor, SARS-CoV-2 and avian H9N2 influenza virus were successfully detected with high-speed and high-sensitivity.

In addition, we found that the control of the surface charge density of graphene is important to get the highly sensitive detection. The sensitivity reaches its maximum when the surface charge density is close to zero. To realize this condition, we propose the new method i.e., the polymer carrying positive charge, PLL, was added onto GFET to make the surface charge density of graphene approach to zero. Using this method, we have succeeded in enhancing 10 times higher sensitivity for the detection of SARS-CoV-2.

Moreover, it was found that the virus can be detected in acetate buffer without cationic polymer. These results obey the theory from the equation combining the formula of Poisson equation and Boltzmann diffusion.

Furthermore, we found that the V_{DP} drift in the bio sensing is attributed to the drift of the leakage current between the reference electrode and source/drain metal electrode. By suppressing this gate leakage current using the SiO_2 passivation layer, we have succeeded in reducing the drift effect in the virus measurement, and consequently, high-speed and high-sensitivity virus detection was successfully achieved.

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List of Publications

Scientific Journals -first author-

1. *“SARS-CoV-2 detection by using graphene FET arrays with a portable microfluidic measurement system”*
Kaori Yamamoto, Natsuki Sato, Kiyoji Sakano, Yasushi Kanai, Shota Ushiba, Naruto Miyakawa, Shinsuke Tani, Masahiko Kimura, Yohei Watanabe, Hidekazu Tanaka, Kazuhiko Matsumoto
(2024 *Jpn. J. Appl. Phys.* 63 016502)
2. *“Great enhancement of sensitivity for SARS-CoV-2 detection by integrated graphene FET biosensor using ζ potential modulator”*
Kaori Yamamoto, Natsuki Sato, Kiyoji Sakano, Mamiko Yano, Eriko Ohnishi, Takao Ono, Yasushi Kanai, Shota Ushiba, Naruto Miyakawa, Shinsuke Tani, Masahiko Kimura, Yohei Watanabe, Koichi Inoue, Hidekazu Tanaka, Kazuhiko Matsumoto
(2024 *Jpn. J. Appl. Phys.* 63 03SP14 **Spotlights 2024, Highlight papers 2024**)
3. *“Effect of poly-L-lysine for graphene FET biosensor in acetate buffer for highly sensitive detection of SARS-CoV-2”*
Kaori Yamamoto, Natsuki Sato, Mamiko Yano, Kiyoji Sakano, Eriko Ohnishi, Shota Ushiba, Shinsuke Tani, Masahiko Kimura, Yohei Watanabe, Kouichi Inoue, Hidekazu Tanaka, Kazuhiko Matsumoto
(2025 *Jpn. J. Appl. Phys.* 64 076501)
4. *“Extremely Stable Virus sensing using Graphene FET with Suppressed Drift Characteristics due to Reduction of Gate Leakage Current by Passivation Layer”*
Kaori Yamamoto, Kiyoji Sakano, Mamiko Yano, Eriko Ohnishi, Shota Ushiba, Shinsuke Tani, Masahiko Kimura, Yohei Watanabe, Hidekazu Tanaka, Kazuhiko Matsumoto
(Manuscript submitted and under review.)

International Conferences -first author-

2023

1. The 23rd International Conference on the Science & Applications of Nanotubes and Low-Dimensional Materials
“INTEGRATED GRAPHENE FET ARRAY FOR HIGH SENSITIVE DETECTION OF NEW CORONA VIRUS WITH AUTOMATED WASHING SYSTEM“
K. Yamamoto, K. Matsumoto
2. 65th electric materials conference 2023 (USA)
“Integrated Graphene FET Array for High Sensitive Detection of New Corona Virus with Automated Solution Exchange System“
Kaori Yamamoto, Natsuki Sato, Kiyoji Sakano, Takao Ono, Yasushi Kanai, Hidekazu Tanaka, Shota Ushiba, Naruto Miyakawa, Shinsuke Tani, Masahiko Kimura, Kazuhiko Matsumoto
3. 2023 International Conference on Solid State Device and Materials
“Great Enhancement of Sensitivity for SARS-CoV-2 Detection by Integrated Graphene FET Biosensor Using ζ -potential modulator“
Kaori Yamamoto, Natsuki Sato, Kiyoji Sakano, Takao Ono, Yasushi Kanai, Shota Ushiba, Naruto Miyakawa, Shinsuke Tani, Masahiko Kimura, Youhei Watanabe, Hidekazu Tanaka, Kazuhiko Matsumoto
4. 36th International Microprocesses and Nanotechnology Conference
“Detection of SARS-CoV-2 in Saliva by Integrated Graphene FET Array“
Kaori Yamamoto, Natsuki Sato, Mamiko Yano, Kiyoji Sakano, Takao Ono, Yasushi Kanai, Shota Ushiba, Naruto Miyakawa, Ayumi Shinagawa, Shinsuke Tani, Masahiko Kimura, Yohei Watanabe, Shinichi Nakakita, Hidekazu Tanaka, and Kazuhiko Matsumoto

2024

1. 66th electric materials conference 2024(USA)
“pH dependence of SARS-CoV-2 Detection using Graphene FET Array Biosensor “
Kaori Yamamoto, Natsuki Sato, Kiyoji Sakano¹, Takao Ono, Hidekazu Tanaka, Shota Ushiba, Naruto Miyakawa, Shinsuke Tani, Masahiko Kimura, Kazuhiko Matsumoto

2. 2024 International Conference on Solid State Device and Materials

“High Sensitive Detection of SARS-CoV-2 by Graphene FET Using Acetate Buffer based on Poisson-Boltzmann Equation“

K. Yamamoto, N. Sato, M. Yano, K. Sakano, E. Ohnishi, S. Ushiba, S. Tani, M. Kimura, Y. Watanabe, H. Tanaka, and K. Matsumoto

3. 37th International Microprocesses and Nanotechnology Conference

“Drastic Reduction of Drift Characteristic in Insulating Coat Graphene FET Biosensor for Detection of Virus“

Kaori Yamamoto, Natsuki Sato, Kiyoji Sakano, Mamiko Yano, Eriko Ohnishi, Shota Ushiba, Yohei Watanabe, Shinsuke Tani, Masahiko Kimura, Hidekazu Tanaka, Kazuhiko Matsumoto

2025

1. 67th electric materials conference 2025 (USA)

“Drastic Reduction of Drift Characteristic in Graphene FET Biosensor by Reducing Gate Leakage Current Using SU-8 Insulating Coat on Source and drain Electrodes“

2. 38th International Microprocesses and Nanotechnology Conference

“Selective Detection of Avian H9N2 Influenza Virus Using Glycan-functionalized Graphene FET Passivated by SiO₂ Layer“

Kaori Yamamoto, Kiyoji Sakano, Mamiko Yano, Eriko Ohnishi, Shota Ushiba, Shinsuke Tani, Masahiko Kimura, Hidekazu Tanaka, Kazuhiko Matsumoto

Domestic Conferences -first author-

第 24 回「関西コロキウム電子デバイスワークショップ」招待講演 (2024)

Kaori Yamamoto, Natsuki Sato, Kiyoji Sakano, Takao Ono, Yasushi Kanai, Shota Ushiba, Naruto Miyakawa, Shinsuke Tani, Masahiko Kimura, Yohei Watanabe, Hidekazu Tanaka, Kazuhiko Matsumoto

2023

1. 第 70 回応用物理学会春季学術講演会

「断片化した抗体を用いた G-FET バイオセンサの開発」

山本佳織、佐藤夏岐、矢野真美子、坂野喜代治、小野堯生、金井康、牛場翔太、宮川成人、品川歩、谷晋輔、木村雅彦、渡邊洋平、中北愼一、河原敏男、鈴木康夫、井上恒一、松本和彦

2. 第 84 回応用物理学会秋季学術講演会

「集積化 G-FET アレイを用いた唾液中の SARS-CoV-2 の検出」

山本佳織、佐藤夏岐、矢野真美子、坂野喜代治、小野堯生、金井康、牛場翔太、宮川成人、品川歩、谷晋輔、木村雅彦、渡邊洋平、中北愼一、田中秀和、松本和彦

2024

1. 第 71 回応用物理学会春季学術講演会

「集積化 GFET アレイを用いた SARS-CoV-2 の検出と ζ 電位変調材の効果」

山本佳織、佐藤夏岐、矢野真美子、坂野喜代治、大西映里子、小野堯生、牛場翔太、宮川成人、品川歩、谷晋輔、木村雅彦、渡邊洋平、田中秀和、松本和彦

2. 第 85 回応用物理学会秋季学術講演会

「絶縁コートによる集積化 GFET のドリフト安定化」

山本佳織、佐藤夏岐、矢野真美子、坂野喜代治、大西映里子、牛場翔太、谷晋輔、木村雅彦、渡邊洋平、田中秀和、松本和彦

2025

1. 第 72 回応用物理学会春季学術講演会

「GFET の参照電極-ゲート電極間のリーク電流の検討」

山本佳織、佐藤夏岐、矢野真美子、坂野喜代治、大西映里子、牛場翔太、谷晋輔、木村雅彦、渡邊洋平、田中秀和、松本和彦

2. 第 86 回応用物理学会秋季学術講演会

「糖鎖機能化 GFET を用いた H9N2 インフルエンザウイルスの検出」

山本佳織、矢野真美子、坂野喜代治、大西映里子、牛場翔太、谷晋輔、木村雅彦、田中秀和、
松本和彦