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Detection of Severe Acute Respiratory Syndrome (SARS) coronavirus nucleocapsid protein using AlGaN/GaN high electron mobility transistors

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AlGaN/GaN high electron mobility transistors (HEMTs) were used to detect the SARS coronavirus (SARS-CoV) nucleocapsid protein interaction without fluorescent labeling. The detection limit in our system was approximately 0.003 nM of protein sample. Our result showed that this technique was more competitive than isotope-labeling EMSA. We demonstrated AlGaN/GaN was highly potential in constructing a semiconductor-based-sensor binding assay to extract the dissociation constants of nucleic acid-protein interaction.

Introduction

The Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) nucleocapsid protein (N protein) plays important roles in forming a nucleocapsid, a viral RNA -protein complex with genomic RNA to ensure the timely replication. The protein encapsulates the viral genomic RNA and ultimately carries its genomic RNA into a host cell. Recently, SARS-CoV N protein has been shown its capability of interacting with RNA, but also with DNA. Thus, SARS-CoV N protein is known as a nucleic acid binding protein[1-7].

Investigating the nucleic acid-SARS-CoV N protein interaction can help us to explore the virus genome packaging process to construct a genome packaging model for SARS coronavirus. Electrophoretic Mobility Shift Assay (EMSA) and Filter binding assay have been widely used to study protein-nucleic acid interaction in the last 30 years[8-14]. However, these two methods require labeling of fluorescent probes or isotope elements on nucleic acids to provide signals for quantitative molecule detection. Thus, the cost for these methods is high, and the labeling may alter the binding affinity of molecules. To develop an efficient and molecule-labeling free binding assay with low cost and high sensitivity becomes a very important issue.

Recently, field-effect transistor (FET) devices have been shown its capability of biomaterial sensing, and also used to measure the dissociation constants of ligand-receptor interactions, such as protein-protein interaction and aptamer-protein interaction[15-19] without fluorescent labeling. Comparing to the EMSA, the FET sensor-based binding assay does not need to extract the free nucleic acids from nucleotide–

protein mixture like in gel electrophoresis. In gel electrophoresis, the size of nucleotide–protein complex was sometimes larger than the pole size of gel[5], resulting to the complex being stuck in gel, which obstruct users to measure the dissociation constants in their system by EMSA.

The cost for manufacturing nanowire-based FET is still high, so this kind of devices could not be efficiently applied to support the biological research immediately. Developing a planar semiconductor-based sensor for biological binding affinity investigation could decrease the cost for detection of biomarkers. Here, we demonstrated using the AlGaN/GaN HEMT-based sensors to detect the SARS-CoV CTD s protein. Also, the size of our devices is in micrometer scale, and it would be suitable for mass production due to the mature technology for semiconductor fabrication.

Experiment

AlGaN/GaN high electron mobility transistors (HEMTs) were used for detecting biomolecule binding such as antibody-antigen, ligand-receptor[20-22]. Here, we demonstrated that the AlGaN/GaN HEMTs also have the capability of detecting nucleic acid-protein interaction with high sensitivity. Figure 1(a) and (b) show the schematics of a DNA-immobilized AlGaN/GaN HEMT sensor and the plan-view microphotograph of the device, respectively. We used the metal-organic chemical vapor deposition (MOCVD) to develop the AlGaN/GaN layers. The structure of the AlGaN/GaN layer consisted of a 3 μm-thick undoped GaN buffer, 150 Å-thick undoped $Al_{0.25}Ga_{0.75}N$ and 10 Å-thick undoped GaN cap layer. The Mesa isolation was performed with an Inductively Coupled Plasma (ICP) etching with $Cl_2/BCl_3/Ar$ based discharges at –90 V dc self-bias, ICP power of 300 W. 10 × 50 μm^2 Ohmic contacts separated with gaps of 5 μm consisted of e-beam deposited Ti/Al/Pt/Au patterned by lift-off and annealed at 850 ºC, 45 sec under flowing N_2.

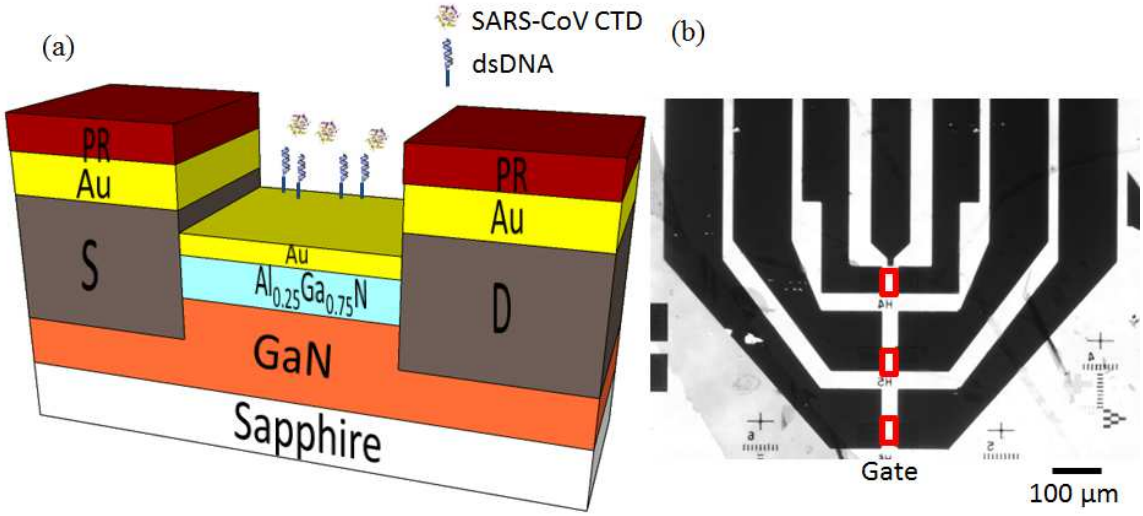

Figure 1. (a) Schematic of the AlGaN/GaN HEMT sensor. (b) Plan view of microphotograph of a completed device.

The dsDNA were the sensing elements, and immobilized on gate regions. The sequence of dsDNA was from SARS-CoV genome[23] (29,580-29621), and conjugated

with a 20 bases poly-dT ssDNA tail. Figure 2 and Figure 3 show the real-time detection of the protein sample at constant bias of 350 mV for the sensor. Phosphate buffered saline (10 mM sodium phosphate pH 6.0, 50 mM NaCl, 1 mM EDTA) was initially dropped on the sensor. There was no net current change until the target protein concentration of 0.003nM of the protein was added. A clear current change was observed as the system reached a steady state. Real-time current monitoring spanned the range of protein concentrations from 0.003 nM to 3000 nM, and showed saturation as the concentration of SARS-CoV CTD larger than 300 nM.

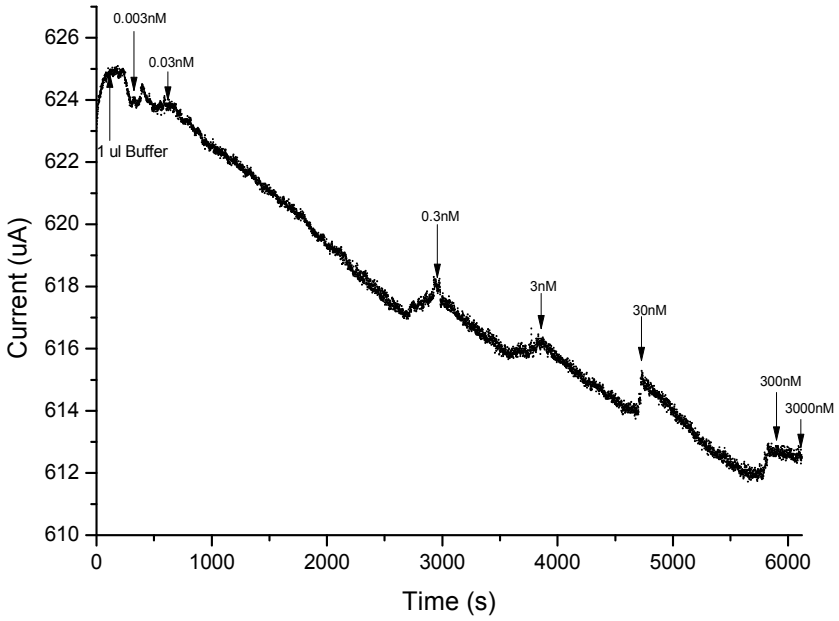

Figure 2. Real-time detection of the SARS-CoV CTDs from 0.003 nM to 3000 nM at constant bias of 350 mV.

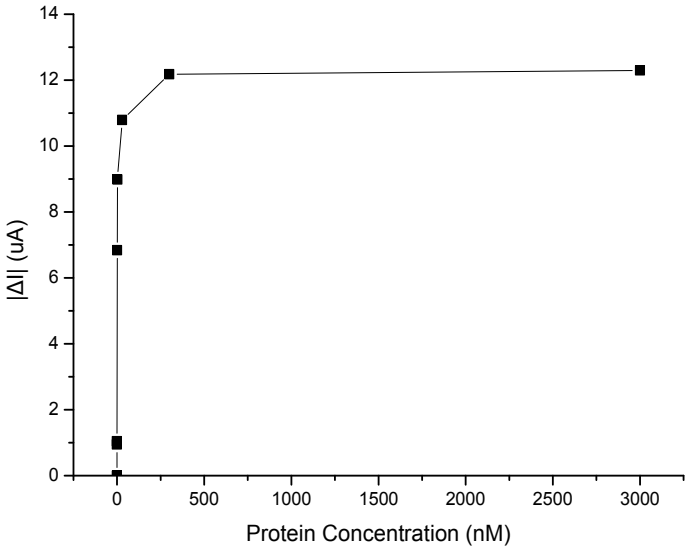

Figure 3. Absolute value of current change with protein concentration from 0.003 nM to 3000 nM.

Result and Discussion

The detection limit of AlGaN/GaN HEMTs sensor for SARS-CoV N protein of dsDNA immobilized device was 0.003 nM, and two orders lower than AMPs immobilized nanowire FET[17]. The amount of SARS-CoV CTDs in 0.003 nM was about 0.4 pg. This result showed the sensitivity of ours system could compete with isotope-labeling EMSA. The usage of protein sample was significantly low, and the amount of SARS-CoV CTDs in once experiment was about 400 ng. Moreover, the salt concentration in our experiment was 50 mM, and was 33 times larger than the salt concentration which used in AMPs immobilized nanowire FET system[19]. Thus, our system was approach to physiological condition, and more suitable for biological application.

Summary

Our result showed the detection limit in our system was 0.003nM. The total amounts of SARS-CoV CTDSs were approximately 0.4 pg, and competed with isotope-labeling EMSA. In summary, AlGaN/GaN HEMTs can detect the nucleic acid binding protein at a low detection limit. Here, The AlGaN/GaN HEMTs was demonstrated to be an efficient tool for biological study, and can potentially extract the biochemical information such as dissociation constants, and chemical dynamics.

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