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General

Investigation of C-terminal domain of SARS nucleocapsid protein–Duplex DNA interaction using transistors and binding-site models

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ABSTRACT

AlGaN/GaN high electron mobility transistors (HEMTs) were used to sense the binding between double stranded DNA (dsDNA) and the severe acute respiratory syndrome coronavirus (SARS-CoV) nucleocapsid protein (N protein). The sensing signals were the drain current change of the HEMTs induced by the protein–dsDNA binding. Binding-site models using surface coverage ratios were utilized to analyze the signals from the HEMT-based sensors to extract the dissociation constants and predict the number of binding sites. Two dissociation constants, K_{D1} = 0.0955 nM, K_{D2} = 51.23 nM, were obtained by fitting the experimental results into the two-binding-site model. The result shows that this technique is more competitive than isotope-labeling electrophoretic mobility shift assay (EMSA). We demonstrated that AlGaN/GaN HEMTs were highly potential in constructing a semiconductor-based-sensor binding assay to extract the dissociation constants of nucleotide–protein interaction.

The breakout of severe acute respiratory syndrome (SARS) in 2002 caused a large mortality and almost paralyzed the world economy in 2003. More than 8000 probable infected cases and 774 deaths were reported worldwide ranging 29 countries, according to World Health Organization (WHO) [1]. This disease is caused by the SARS coronavirus. A coronavirus protein, nucleocapsid protein (N protein), encapsidates the coronavirus genomic RNA playing an important role in the virus replication. SARS-N protein has been shown its capability of interacting with RNA and DNA, and therefore is known as a nucleic acid binding protein [2–5]. Investigating the nucleotide-SARS-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 [6–9]. 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. Developing an efficient and molecule-labeling free binding assay with low cost and high sensitivity becomes a very important issue.

AlGaN/GaN high electron mobility transistors (HEMTs) have been successfully demonstrated for detection of gases, chemicals, and biomolecuels [10–17]. The AlGaN/GaN HEMT sensors have shown high sensitivity due to the high concentration of two-dimensional electron gas induced by the piezoelectric polarization of the strained AlGaN layer and the spontaneous polarization [10,15,17]. The gate region of the HEMTs can be modified or immobilized with various sensing materials to interact with analytes, which result in potential change on gate area leading to the current change of transistors. Gallium nitride (GaN) is thermally stable because of the wide-bandgap property. Devices made of GaN can

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operate up to 500 °C. GaN is also chemically stable. Most acids and bases cannot etch GaN in regular condition. Only molten NaOH or KOH is known to be able to etch GaN. Therefore GaN-based sensors are particularly suitable for detection with salt, ions, chemicals, or biomolecules in liquid. The AlGaN/GaN HEMT-based sensors are excellent candidates for detection under harsh environments such as in buffered solution, serum, or in sea. In addition to the good thermal and chemical stability, the AlGaN/GaN HEMT has advantages such as real-time detection, fast response, high sensitivity, small size, light-weight, low cost and good reliability. These advantages ensure the AlGaN/GaN HEMT-based sensors to be an excellent platform to investigate the binding affinity of ligand–receptor complexes.

The AlGaN/GaN HEMTs are not only can be used as biosensors to detect various biomolecules, but can also be used to study the binding affinity of ligand–receptor complexes. The ligand–receptor pairs may include antigen-antibody, DNA–DNA, protein–DNA, protein–RNA, protein–protein, drug–protein, or substrate/inhibitor–enzyme systems. On the gate region of the HEMT sensors, receptors are usually immobilized to detect the ligand concentration in the bulk solution. The sensing mechanism is through the ligand–receptor affinity, which can be quantitatively expressed as equilibrium constant (affinity constants) or dissociation constants. Besides, a receptor may bind with more than one ligand, and each binding site on the receptor may have a unique dissociation constant with the ligand. With a quantitative analysis of the signals from the sensors through binding-site models, we are able to reveal how many binding sites are on the receptor (dsDNA) for certain ligands (SARS-N protein) and what the dissociation constants are for ligand–receptor complexes. Due to the fast response and low price of the AlGaN/GaN HEMT sensors, the analysis can be quickly done and the information about the ligand–receptor binding affinity can be obtained with very low expenses. Conventional methods to study ligand–receptor interactions also include enzyme-linked immunosorbent assay (ELISA) [18], isothermal titration calorimetry (ITC) [19,20], ultra-violet/visible light (UV/VIS) spectrum [21], surface plasmon resonance (SPR) [22,23], total internal reflection ellipsometry (TIRE) [24], and high-performance liquid chromatography (HPLC) [25]. Typically, ELISA and UV/VIS spectrum require labeling probes. ITC usually needs a large amount of sample. The commercial equipment (BIAcore) utilizing SPR technology has been used to characterize binding affinity of ligand–receptor complexes [17]. However, the equipment is expensive and therefore the cost for research is high. TIRE or HPLC also have good performances, but their prices are much higher than that of AlGaN/GaN HEMT sensors. In sharp contrast, the AlGaN/GaN HEMT sensors can achieve very good performances as the above mentioned while in the meantime keep a very low cost for research.

To investigate the number of binding sites and the dissociation constants of a receptor, the surface coverage ratio is adopted in one-binding-site model or multiple-binding-site model. The surface coverage ratio means the ratio of the number of ligand-bound receptors to the total number of receptors on the sensor. The ratio is related to the bulk concentration of the ligand, which is associated with the magnitude of the signals from the sensor. Dissociation constants are extracted by fitting experimental results into the binding-site models, and the number of binding sites on the receptors is obtained.

In this study, SARS-N protein–dsDNA binding pair is studied in the binding affinity with the theoretical binding-site models and the experimental results. One-binding-site mode and two-binding-site model are fitted with experimental results. The analysis of the signals from AlGaN/GaN HEMT sensors using the binding-site models demonstrates that the AlGaN/GaN HEMTs cannot only be used as biosensors, but also can be used to study the biological binding affinity of ligand–receptor pairs. Here AlGaN/GaN HEMTs were immobilized with dsDNA on gate region as the receptors for binding SARS-N proteins. The dsDNA is a 41-base dsDNA sequence from SARS coronavirus genome (base pairs 29,580 to 29,621) [5,26] which is conjugated to a 20-mer poly-dT single stranded DNA (ssDNA) tail to its 3′-end in one of the single strand of the dsDNA. The SARS-N protein can be divided into two binding domains, the C-terminal domain (CTD) and the N-terminal domain (NTD). Both the two domains can bind with nucleic acids through their electropositive region. In this study, the SARS-N protein that we used has the CTD only. The sensors were used to study the affinity between the CTD and the dsDNA.

Methods

Fabrication of AlGaN/GaN HEMTs

The HEMT structure 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 AlGaN layer was designed to induce high spontaneous polarization, which generated a two-dimensional electron gas beneath the AlGaN layer. The epilayers were grown by metal–organic chemical vapor deposition (MOCVD) on sapphire substrates. Mesa isolation was performed using an inductively coupled plasma (ICP) etching system with Cl_2/BCl_3 gases under ICP power of 300 W at 2 MHz and a process pressure of 10 mTorr. Ohmic contacts ($60 \times 60\ \mu m^2$) separated with gaps of 30 μm consisted of e-beam deposited Ti/Al/Ni/Au and was annealed at 850 °C, 45 s under flowing N_2. 100 Å-thick gold was deposited on the gate region. Photoresist of 1.8 μm (Shipley S1818) was used to encapsulate the source/drain regions, with only the gate region open to allow the liquid solutions to cross the surface.

DNA surface immobilization

The ssDNA molecules 5′-thiol-TTTTTTTTTTTTTTTTTTTTTTCATCGAGGCCACGCGGAGTACGATCGAGGGTACAGTGAA-3′, and 3′AAGTAGCTCCGGTGCGCCTCATGCTAGCTCCCATGTCACTT-5′) were purchased from Genomics (Taipei, Taiwan). The dsDNA was hybridized by these two ssDNA molecules in TE Buffer (30 mM Tris–HCl, pH = 8.0, 30 mM NaCl, 1 mM EDTA, 25 mM TCEP (tris(2-carboxyethyl)phosphine). Then, the dsDNA solution was dropped on the gate area of AlGaN/GaN HEMTs, and waited for 30 h. The free dsDNA was washed away with phosphate buffer (10 mM sodium phosphate, pH = 6.0, 50 mM NaCl, 1 mM EDTA).

Sensor measurement

The characteristics of source–drain current–voltage of the sensor was measured at 25 °C using an Agilent B1500 parameter analyzer with the gate region exposed. The source-drain bias was fixed at 0.35 V. Different concentrations of the SARS N-protein CTD were sequentially dropped on the sensor in a real-time measurement.

Results and discussion

Fig. 1(a) and (b) shows the schematics and the plan-view of the SARS-N protein sensor, respectively. Fig. 2 shows the real-time measurement of the SARS-N protein 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 is no net current change until the target protein concentration of 0.003 nM of the protein was added. A clear current change was observed as the system reached a steady