AlGaIn/GaN heterostructure field transistor for label-free detection of DNA hybridization

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This paper demonstrates electrical detection of single strand deoxyribonucleic acid (ssDNA) conjugation by AlGaIn/GaN heterostructure field effect transistor (HFET) biological sensors. The probe ssDNA molecules are modified by thiol groups. The immobilization of probe molecules is achieved by S–Au bonding on a thin layer of gold film in the sensing area. The immobilization and hybridization process are firstly implemented on Si surfaces and checked by fluorescent and atomic force microscopy (AFM) imaging. The hybridization process is monitored on AlGaIn/GaN HFETs. Time-dependent current change is observed when a matched ssDNA solution is applied, while no response is observed for a mismatched ssDNA sequence. The DNA hybridization process is dominated by the conjugation between matched ssDNA sequences in the first few tens of seconds. After that, the hybridization process is dominated by mass transfer processes and saturation of the immobilized probe ssDNA molecules.

AlGaIn/GaN HFETs, biological sensors, surface modification, hybridization, AFM

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Biological sensors are of great research interests nowadays. Biological sensors have many applications, one of which is to detect agents for biological or chemical weapons of mass destruction (bio/chem. WMD) in battle field situations [1] and against bioterrorism to save lives. For this purpose, biological sensors should be light and easy to carry, with high sensitivity. Biosensors can also be used to detect viruses, DNA, etc., for disease diagnostics and control, new medicine development, and drug delivery etc. [2].

There are several ways to detect the presence or conjugation of biological molecules such as surface plasmon resonance (SPR) [3], quartz crystal microbalance (QCM) [4], biological micro(nano)-electro-mechanical system (BioMEMS/BioNEMS) [5], bio-barcode assay (BCA) [6,7], fluorescent imaging [8], and electrical biosensors [9–11]. Each of these technologies has its own advantages. However, their appli-

cations are limited by their drawbacks. SPR and optical biosensors are based on fluorescence, which can be very sensitive, but cannot be used in turbid media. Nanomechanical oscillators and QCM cannot be used for real-time monitoring since the devices need to be removed from the solution during the measurement. Electrical biosensors have been found to have the capability of detecting biomolecules for real-time applications. Different material systems have been developed for this purpose. Si nanowires [9], carbon nanotubes [10], and AlGaIn/GaN heterojunction field-effect transistor (HFETs) [11] have been demonstrated to be able to detect biological target molecules such as DNAs and proteins with low concentrations during the process of conjugation. Device structures are simple, and the measurement setup is easy to minimize for integration.

In this paper, real time electrical detection of single-strand DNA (ssDNA) hybridization is demonstrated on an AlGaIn/GaN HFET structure.

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1 AlGaN/GaN biosensor

1.1 Introduction to AlGaN/GaN heterostructure

AlGaN/GaN heterostructure is known to be suitable for optoelectronic and microwave power devices because of large bandgap, large electron saturation velocity, and high thermal stability [12]. Two-dimensional electron gases (2DEG) with a sheet carrier concentration on the order of 10^{13} cm^{-2} is achievable without intentional doping in AlGaN/GaN structures. In such structures, there is spontaneous polarization in equilibrium lattice and piezoelectric polarization induced by the strain between AlGaN and GaN. The 2DEG is introduced by the discontinuity of polarization at the AlGaN/GaN interface and it is highly sensitive to proximal charges. Positive surface states accumulate electrons in 2DEG channel and therefore increase the current between source and drain (I_{ds}), while negative surface states behave oppositely.

1.2 AlGaN/GaN heterojunction field effect transistor (HFET) biosensors

Unlike GaAs or other III–V materials, surface states do not pin surface Fermi level strongly for AlGaN/GaN heterostructures. As a result, the presence of charged molecules or particles on AlGaN/GaN surface will cause a change of the Fermi level. Hence, the 2DEG sheet carrier concentration in the channel can be modulated correspondingly. This change can be extracted by the capacitance-voltage (C-V) measurements of AlGaN/GaN HFETs. It has been shown that AlGaN/GaN based HFETs or Schottky diodes are appropriate for detection of hydrogen gases [13], polar liquid [14], proteins [15], and DNAs [11].

Figure 1 shows a typical carrier concentration profile of the AlGaN/GaN structure used in this study, which is extracted from Schottky diodes by C-V measurements. As one can see, most of the 2DEG electrons are in a quantum well and the distance to the surface is on the order of a few tens of nanometers (typically around 20 nm). Hence, the change of surface potential will modulate the concentration of electrons

Figure 1 C-V concentration profile as a function of depth of AlGaN/GaN heterostructure.

confined at the AlGaN and GaN interface.

The binding rate between immobilized probe molecules on the solid surface and target molecules in solution should theoretically be positively correlated with the concentration of target molecule solution. For AlGaN/GaN HFETs, since the gate area can be large, the binding events cannot happen within a very short period of time, which means that the binding rate can be calibrated. Therefore, planar AlGaN/GaN HFETs are a good platform to study the binding kinetics.

1.3 Sensor selectivity and surface modification of AlGaN

To immobilize different probe molecules, the AlGaN surface needs to be modified to link receptor molecules for specific binding or hybridization to target biomolecules. A commonly used method is to oxidize AlGaN surface and silanize the surface oxide by O–Si binding. Silane molecules can have bioaffinity groups such as amine groups. This method has been used to immobilize biotin probes [16,17]. Another way is by depositing a thin layer of metal film where atoms in the deposited film can bind with certain groups in biomolecules to achieve immobilization. In this study, Au films are coated on the sensing area for DNA assembly and immobilization [18].

The Debye length in water is on the order of angstroms, beyond which the presence of target biomolecules cannot be detected by electrical sensors. So minimization of the distance between target biomolecules and AlGaN surface is critical. For this purpose, Au–S binding was selected to modify AlGaN/GaN HFETs. Since metal layers can shield adsorbed charges on the surface and also have an impact on the surface donors. The thicknesses of metal layers were designed to be thin.

1.4 Device structure and fabrication

The AlGaN/GaN heterostructures used in this study were grown by metal organic chemical vapor deposition (MOCVD) on a (0001) sapphire substrate. A 40 nm AlN nucleation layer was first grown on the sapphire substrate, followed by a 3 μm undoped GaN layer. A 20 nm undoped Al_{0.3}Ga_{0.7}N layer was grown on the GaN layer to form the heterostructure. Hall measurements at room temperature showed a sheet carrier density of $1.3 \times 10^{13} \text{ cm}^{-2}$ and an electron mobility of $1175 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$.

The cross-sectional view of devices is shown in Figure 2. The electrical isolation between devices was performed by Cl₂, BCl₃, and Ar plasma dry etching process using an inductively-coupled plasma (ICP) Oxford PlasmaLab100 system. A liftoff process of Ti (350 Å)/Al (900 Å)/Mo (600 Å)/Au (1000 Å) layers was implemented to define Ohmic patterns. Ohmic contacts were formed by annealing at 850°C for 30 s in a rapid thermal annealing (RTA) system

in N_2 ambient. The electrodes were isolated from the solution by a polyimide layer patterned by optical lithography. The polyimide thickness is around 10 μm after curing at 320°C for 1 h. The size of gate area is on the order of hundreds of micrometers. After that, Ti/Au was deposited as the immobilization area for thiol-modified ssDNA probes. The Ti layer is used to increase the adhesion between gold and AlGaIn surfaces. The thicknesses of Ti and Au are 10 and 50 Å, respectively. After the gate metal deposition, the current levels of the devices were observed to decrease by around 10% due to band bending and Fermi level shift.

2 Experiments and results

2.1 ssDNA immobilization and hybridization on Au surfaces

The processes of immobilization and hybridization of ssDNA on Au surfaces were first studied by fluorescent imaging and atomic force microscopy (AFM) measurements before they were transferred to AlGaIn/GaN surfaces. The study was performed on Si substrates with Au patterns. The 12 mer de-salted oligonucleotides (Sigma-Aldrich) were used in this study. Table 1 shows the sequences of probe and target ssDNAs. There are two copies of each specific sequence, one with fluorescent labeling and another without. Here p1 and t2 are matched ssDNA sequences, while t1 is mismatched sequence to p1 for control study. TE buffer with pH 8.0 was used for hybridization tests.

Fluorescent imaging was implemented on a Nikon ECLITSE TE2000-S fluorescent microscope. The exposure time was set to 5 s. The fluorescent images are shown in

Figure 2 (Color online) Cross-sectional view of AlGaIn/GaN HFET biosensors.

Table 1 Oligonucleotide sequences and nomenclature for electrical detection and fluorescent imaging

DNA type	Abbreviation	Sequences
Probe	p1	5'-CCT AAT AAC AAT 3'-C2-thiol
Probe	fp1	Cy5-5'-CCT AAT AAC AAT 3'-C2-thiol
Target	t1	5'-AAA AAA AAA AAA-3'
Target	ft1	Cy3-5'-AAA AAA AAA AAA-3'
Target	t2	5'-ATT GTT ATT AGG-3'
Target	ft2	Cy3-5'-ATT GTT ATT AGG-3'

Figure 3. The left side of each picture shows the fluorescent images with a sampling bar. The right side shows the fluorescent intensities vs position across the sampling bar. The fluorescent images were taken under aqueous condition. Figure 3(a) shows the background emission signal on gold and silicon under the illumination of 649 nm wavelength light (excitation wavelength of Cy5). The sample was first rinsed in 1 mmol L^{-1} fp1 solution for 12 h, washed by water and taken out for imaging. Figure 3(b) shows a significant increase in fluorescence intensities due to Cy5 emission

Figure 3 (Color online) Fluorescent images of Si substrates with Au patterns in the process of ssDNA immobilization and hybridization. (a) Background image of Cy5 excitation wavelength, (b) image after probe ssDNAs immobilization, (c) image of Cy3 excitation wavelength with probe ssDNA immobilized, (d) image after treated with mismatched ssDNA solution, and (e) image after treated with matched ssDNA solution.

under illumination, indicating that the probe ssDNA molecules have bonded to the Au surface.

After that, the sample was rinsed in 1 mmol L⁻¹ mercaptoethanol (MCE) solution for 2 h to block the area without any probe ssDNA molecules to avoid physical adsorption of target ssDNAs and help probe ssDNAs to have better conformation for hybridization. Followed by that, the substrate was washed in water and cleaved into two pieces. One piece was rinsed in 1 mmol L⁻¹ ft1 solution and the other piece was rinsed in 1 mmol L⁻¹ ft2 solution. There is 1 mol L⁻¹ NaCl in target ssDNA solution to improve hybridization. The two samples were taken out after 1 h incubation, washed by water and taken fluorescent images. Figure 3(c) shows the background emission signal on gold and Si with the excitation wavelength of Cy3 at 550 nm. Figure 3(d) and (e) show the fluorescent intensities of the sample rinsed in ft1 and ft2 separately. By comparing the fluorescent intensity changes, the increase in fluorescent intensities on the ft2 solution rinsed sample confirms that hybridization indeed happened, while the fluorescent intensities on the sample rinsed in ft1 solution are only slightly higher than the background intensity level.

The AFM images of Au surfaces during the immobilization and hybridization process are shown in Figure 4. The grain sizes in Figure 4 confirm the immobilization and hybridization of ssDNA molecules. In Figure 4(a), there are no circular grains on the Au surface, and the feature pattern sizes are small, which is the initial condition for ssDNA molecules to attach. Figure 4(b) shows the Au surface morphology after the sample was rinsed in 1 mmol L⁻¹ probe ssDNA solution for 12 h. Circular grains appeared all over the Au surface due to the binding of probe ssDNAs on the Au surface. Figure 4(c) shows that the circular grain sizes were increased further by the hybridization of target ssDNA

Figure 4 (Color online) AFM images of Au surfaces (a) after Ti/Au deposition, with a surface roughness of 0.989 nm, (b) after probe ssDNA immobilization, with a surface roughness of 1.265 nm, and (c) after hybridization with matched target ssDNAs with a roughness of 1.172 nm.

molecules. The surface roughness of the Au surfaces did not change significantly after the binding of probe ssDNAs, and hybridization of target DNAs with a matched sequence, indicating that the uniformity of SAM is good.

2.2 Electrical detection

After the surface modification and immobilization/hybridization processes were developed on Si surfaces, they were implemented to AlGaIn/GaN HFETs for electrical detection. After the device fabrication, the sample was rinsed in 1 mmol L⁻¹ p1 solution for 12 h, and followed by a rinse in 1 mmol L⁻¹ MCE solution for 2 h. The device was then biased at 0.5 V, and the current was monitored using an Agilent 4156C semiconductor parameter analyzer. Comparison measurements were performed by applying a 1 mmol L⁻¹ t1 solution and t2 solution to two different devices, respectively.

Figure 5 (a) shows the current versus time plot during the hybridization process with matched ssDNA target. The hybridization was not observed until around 150 s after the target ssDNA solution was applied, which was also observed by Kang et al. [11]. The 150 s delay is due to the diffusion process of the target DNA molecules onto the device surface, and the reconfiguration process of their positions when they get close to the sensing surface before hybridization. During these processes, there is no current change or charge transfer as the hybridization has not taken

Figure 5 Time-dependent current responses of AlGaIn/GaN HFETs with probe ssDNAs on Au-modified gate to (a) matched and (b) mismatched ssDNA solution.

place. For the hybridization process, as shown in Figure 5(a), in the first few tens of seconds, the decrease of current is sharp and most of the current change happens during this period. In this time period, there were enough target ssDNA molecules to hybridize with the surface probes, which indicates that the current change rate is dominated by the ssDNA hybridization process. As the target ssDNA molecules in the surface area being consumed by hybridization, the current change rate was dominated by mass transfer process for target ssDNA molecules to diffuse to the surface area. As all the probe ssDNA molecules on the Au surface are hybridized with target molecules, the current change rate saturates. The three processes discussed above determine the hybridization rate in the short time range (e.g. first few tens of seconds), the middle time range, and the long time range, though the last two processes are difficult to isolate in the current-time dependent measurements. The current change after hybridization, or current saturation, is about 38 μA . If we define sensitivity as the ratio of current change and base current ($\Delta I/I$), the sensitivity of this device is 1.4%. Figure 5(b) shows the control measurement with mismatched target ssDNA solution applied. There is no appreciable current change observed, which indicates that the hybridization and physical adsorption of target ssDNA molecules to the modified Au surface is minimal and the hybridization process is highly specific. The current variation is less than 4 μA . It should be noted that the difference in baseline current between measurements is mainly caused by the variation of individual devices measured in this work, which is a typical problem for current GaN FET technology before it gets more mature. The device uniformity and reliability issue is beyond the scope of this work, and it is currently being investigated by both industries and academia. Nevertheless, it is important to point out that given the existence of such variation, using $\Delta I/I$ as the sensitivity instead of the absolute current value for such sensor devices is a better way to represent the changes upon DNA hybridization, though the absolute current change is about 10 times larger than the mismatched control case.

3 Conclusions

A design of AlGaIn/GaN HFET biosensor was demonstrated for detection of ssDNA hybridization. The probe ssDNA was modified by thiol groups, and was immobilized on Au surface. The target ssDNA molecules were then hybridized with immobilized probe ssDNA molecules. The hybridization process was studied by both fluorescent imaging and AFM morphology. The immobilization and hybridization process was implemented on functionalized AlGaIn/GaN HFETs. The decrease of current in AlGaIn/GaN HFET bio-

sensors was observed during the hybridization process. In the first few tens of seconds, the hybridization process was dominated by the conjugation between matched ssDNA sequences. After that, the hybridization process was dominated by the mass transfer process of target ssDNA diffusion toward to the sensing surface before the current saturation.

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