Mater. Res. Soc. Symp. Proc. Vol. 1202 © 2010 Materials Research Society 1202-I09-09

Detection of DNA hybridization using functionalized InN ISFETs

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ABSTRACT

Ultrathin (~10 nm) InN ion sensitive field effect transistors (ISFETs) are functionalized by immobilized label-free oligonucleotide probes with 3-mercaptopropyltrimethoxysilane (MPTMS) through molecular vapor deposition (MVD) technique. This layer on the InN surface serves the function of selectively detecting the hybridization of complementary deoxyribonucleic acid (DNA). Using MVD technique to perform the gas-phase silanization of MPTMS provided a time-saving and simple method to reach 68° water contact angle after 1.5 h treatment. High resolution X-ray photoelectron spectroscopy (HRXPS) was employed to analyze the surface characteristics after functionalization. Modified probes DNA were covalently bonded to MPTMS-covered gate surface of InN ISFETs. And further hybridized with complementary DNA For a 12-mer oligonucleotide probe, a significant drain-source current decrease (~ 6 μA) was observed for the hybridization with complementary DNA solution of 100 nM. In contrast, the noncomplementary DNA with single-base mismatch did not show obvious current changes. Functionalized ultrathin InN ISFETs for DNA sequence detection demonstrate the promise of biological sensing and genetic diagnosis applications.

INTRODUCTION

Deoxyribonucleic acid (DNA) sequence can naturally hybridize with its complementary sequence due to the specific base-pairing rule. This useful property renders DNA probes can be used to detect genetic disease. There have been a variety of methods adopted to detect the DNA hybridization [1-4]. However, these approaches, normally, require prelabeling steps, time-consuming optical measurements, or expensive instrumentation. To pursue label-free, rapid and accurate electrical detection, sensors based on semiconductor materials employing field effect transistor structure have been widely studied [5-7]. In particular, ultrathin InN ion sensitive field effect transistors (ISFETs) have attracted a lot of attention to be biosensors due to high sensitivity, biocompatibility and robust surface stabilities [8]. This purpose, however, requires a proper functionalization for obtaining the desired molecular recognition ability. To date, gas phase preparations have shown can provide better surface modification quality [9]. Molecular vapor deposition (MVD) is one kind of novel and effective coating technology to modify the surface due to a superior capacity of precisely controlling the process conditions [10].

In this work, we functionalize ultrathin InN ISFETs using label-free single strand DNA (ss-DNA) probes which are immobilized by 3-mercaptopropyltrimethoxysilane (MPTMS) molecules through MVD technique. High resolution X-ray photoelectron spectroscopy (HRXPS) and

contact angle measurements are adopted to characterize the surface properties. Electrical measurement is performed to investigate the hybridization with complementary DNA targets.

EXPERIMENT

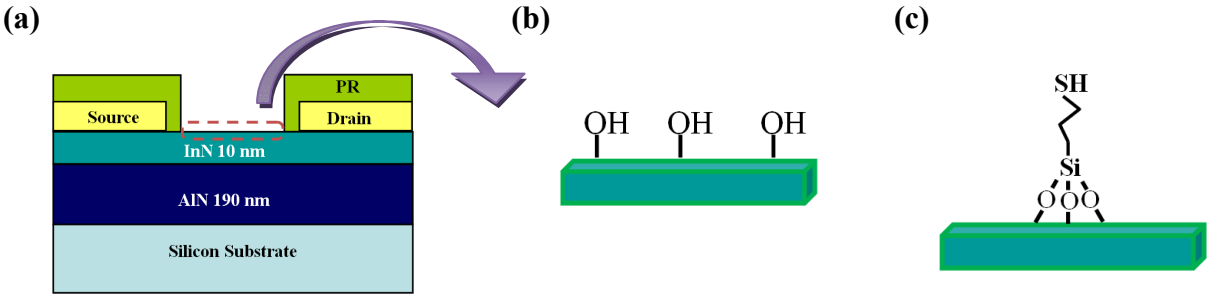
Materials

Adopted InN film as the sensing element was heteroepitaxially grown on a Si(111) substrate via a molecular-beam epitaxy (MBE) system. The InN/AlN film is composed of an ultrathin (10 nm) InN epilayer and a thick (190 nm) AlN buffer layer. Fabrication of InN ISFETs was started from evaporation of Au/Ti (150/80 nm) for obtaining Ohmic contact. Using lift-off process defined the channel length and contact pads of ISFETs. Mesa isolation was performed by inductively coupled plasma (ICP) to define the channel width of ISFETs. Eventually, polyimide was patterned to define the ISFET contact (gate) windows. After microfabrication process, wafer was diced and then ISFETs were bonded onto printed circuit boards using aluminum wires, followed by packing with polydimethylsiloxane (PDMS). The detailed growth process of InN film and fabrication of InN ISFETs can be found elsewhere [8, 11].

The organosilane MPTMS was purchased from Sigma-Aldrich. The adopted oligonucleotides were purchased from MDBio, Inc. The probe DNA modified at the 5' end is acrylic phosphoramidite-$(CH_2)_6$-5'-CCT AAT AAC AAT-3'. The 12-mer complementary DNA and noncomplementary DNA are 5'-ATT GTT ATT AGG-3' and 5'-ATT GTT ACT AGG-3', respectively. All water used was deionized (DI) water with a resistivity 18 MΩ.

Functionalization of InN ISFETs

Prior to O_2 plasma treatment, UV adhesives (Letonnd 5802) were used to encapsulate the fabricated InN ISFETs except for the open gate region. The O_2 plasma with 10 min treatment was performed to clean the samples surface and form a hydroxyl-terminated surface as well. Gas-phase silanization of MPTMS using MVD system was conducted for 1.5 h subsequently. MVD system purged MPTMS gas for continual three 0.5 h cycles. Each cycle comprised eight times purges which were set at 2 torr for each purge. MPTMS molecules were covalently bonded to the gate surface of InN ISFETs and left thiol (-SH) terminal groups to further immobilize probe DNA. MPTMS-covered ISFETs were dipped into 10 μM solution of probe DNA for 12 h. All DNA solutions were prepared by DI water. Furthermore, the experimental samples were rinsed carefully with DI water. After immobilization of probe DNA, the functionalized InN ISFETs were employed to detect the hybridization with complementary ss-DNA.



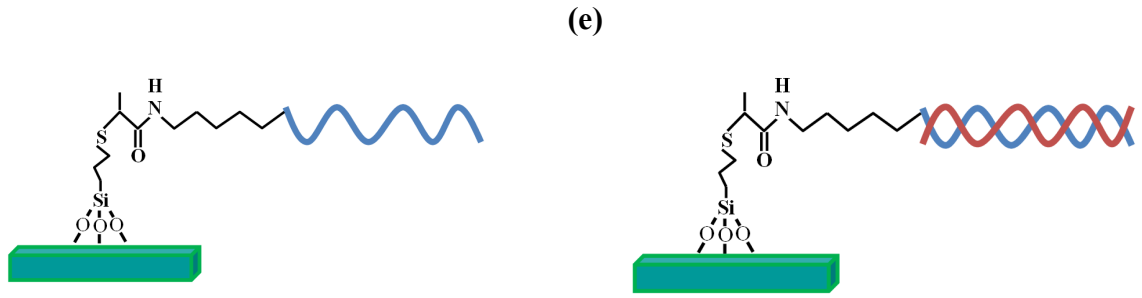


Figure 1. Modification scheme of functionalizing ultrathin InN ISFETs for the detection of DNA hybridization: (a) Fabricated ultrathin InN ISFET; (b) Hydroxylation of InN surface; (c) modification using gase-phase MPTMS through MVD technique; (d) immobilization of probe ss-DNA; (e) hybridization with complementary ss-DNA.

Instrumentation

The gas-phase modification on the gate region of ultrathin InN ISFETs was carried out by MVD system (Applied Microstructures, MVD 100). Contact angle goniometer (Sindatek, Model 100SB) was used to measure the water contact angle at room temperature for investigating the surface properties. High resolution X-ray photoelectron spectrometer (HRXPS) was used to analyze the surface characteristics on the open-gate region of InN ISFETs with approximate 60 μm channel length. HRXPS (ULVAC-PHI, PHI Quantera SXM) was equipped with monochromatic 1486.6 eV X-ray source from the Al Kα line. The incident angle was 45^{o} measured from the sample surface and the electron pass energy was 55 eV. Electrical measurement was conducted by a DSP Lock-in amplifier (Stanford Research System, SR830). A 200 mV Vpp at 1k Hz voltage source (V_{SD}) was applied via a function generator (Agilent, 33250A) to InN ISFET. One standard resistor of 47.7 Ω was in series with InN ISFET because of the consideration of impedance match for better measurement results. Reference electrode $HgCl/Hg_2Cl_2$ (Hanna, HI-5412) was kept at a zero potential during the real-time measurement.

DISCUSSION

Surface Characterization of Functionalized ISFETs

The extent of MPTMS silanization can be investigated by water contact angle. The change of water contact angle on InN surface was observed from 3^{o}, indicating the O_2 plasma cleaning, to 68^{o} after 1.5 h reaction with MPTMS vapor, which is closed to the reported saturation contact angle (~70^{o}) of MPTMS silanization [12]. Moreover, we adopted HRXPS to confirm the surface characteristics of functionalized InN ISFETs. Two different samples were prepared by the following conditions. One was used as a control sample without any modification (Without Modification). The other sample was a complete functionalied ISFET through MPTMS modification and probe DNA immobilization (With Probe DNA + MPTMS). XPS spectrum demonstrates that samples With Probe DNA + MPTMS show the S 2*p* peak at 162.4 eV (Figure 2a). This result reveals the presence of sulfur (S) from MPTMS linkers on InN surface and S 2*p* band indicate other oxidized state of S species. In contrast, no S 2*p* peak is observed for the sample Without Modification. To investigate the probe DNA immobilization, the spectrum of P

atoms was measured (Figure 2b) due to DNA molecules possessing phosphate functional group. The clear P 2*p* peak was only observed from sample (With Probe DNA+MPTMS), which supports the confirmation of probe DNA being immobilized by silane coupling agent successfully.

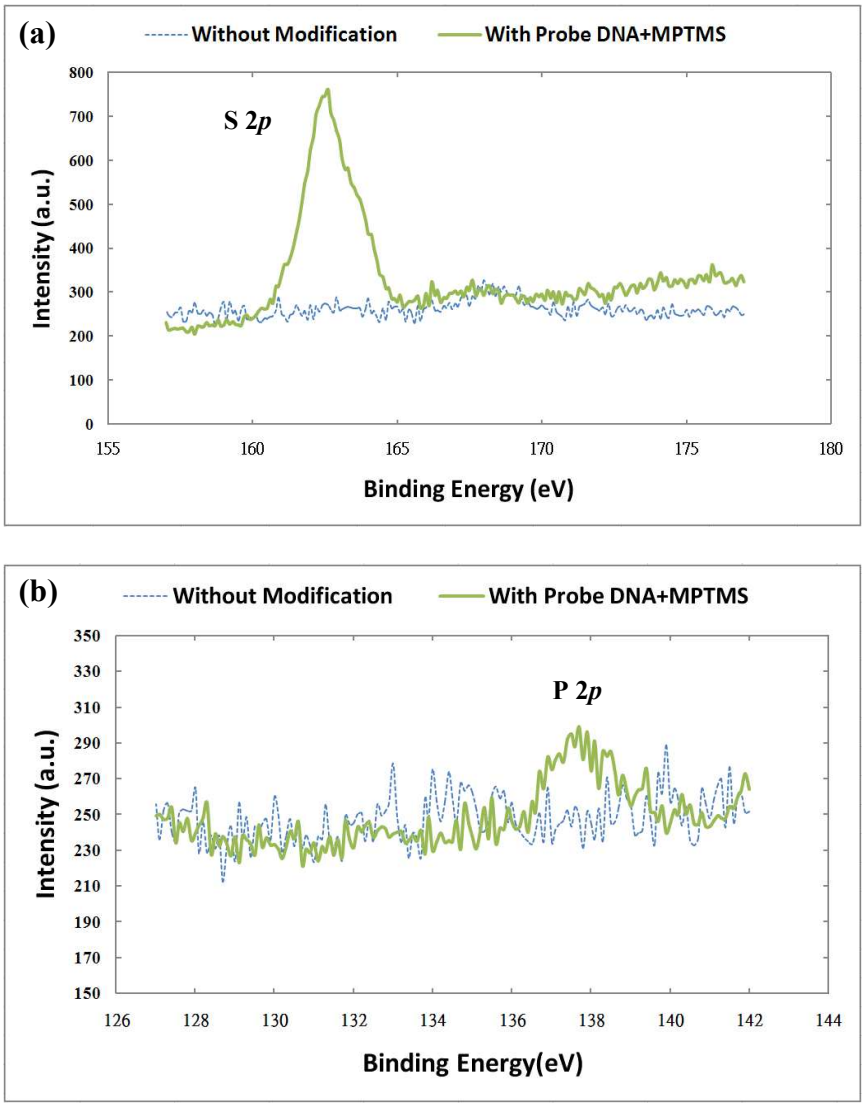


Figure 2. HRXPS spectra of (a) S 2*p* region, and (b) P 2*p* region analyzed on the gate regions of InN ISFETs based on different treatments of without modification (dotted line) and functionalization with probe DNA and MPTMS (solid line).

Electrical Detection of DNA Hybridization

Measuring the dynamic current response of ISFETs can reveal the effect of bio-conjugation such as DNA hybridization due to the charge-based sensing. To minimize the effect of counter ions screening, DI water was used to prepare the DNA solutions for obtaining higher sensitivities. During the sensing, only the gate region of InN ISFET has to be exposed to the aqueous solution. Thus, a complete encapsulation is particularly important to provide good electric insulation as

well as high resistance to water penetration. Figure 3 displays the time dependence of the current response for functionalized InN ISFET on exposure to DNA solutions at approximate 100 s. For control experiment, there was no obvious current change for noncomplementary DNA solution of 100 nM (Figure 3a). On the other hand, a complementary target DNA solution of 100 nM resulted in a significant drain-source current decrease of approximate 6 μA, which current variation was 0.74% (Figure 3b). This result suggested that the attachment of negatively charged complementary DNA with the immobilized probe DNA caused the depletion of the carrier concentration in the ultrathin InN surface so that drain current decreased.

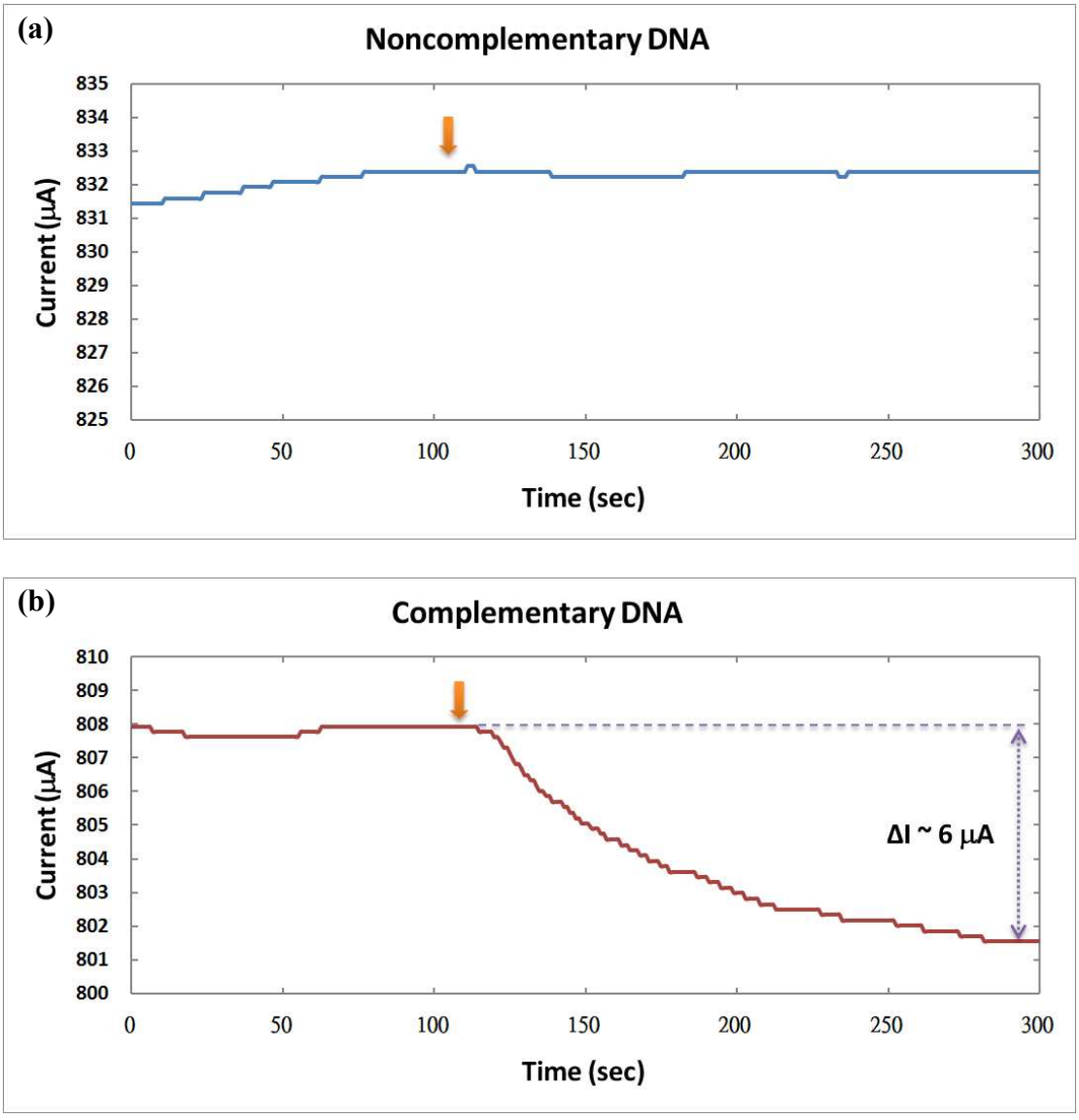


Figure 3. Real-time current responses of functionalized ultrathin InN ISFETs. (a) Response to noncomplementary DNA; (b) Response to complementary target DNA, where arrow represents the injection of DNA solution at ~100 sec to InN ISFETs in DI water.

CONCLUSIONS

Label-free and sequence-specific DNA sensors have accomplished using functionalized ultrathin InN ISFETs. We demonstrate the functionalization on InN surface by immobilizing probes DNA with gas-phase silanization of MPTMS through MVD technique. MVD technique provides a simple, time-saving and effective approach to surface modification with batch processing. Functionalized InN ISFETs with covalently immobilized ss-DNA probes could observe the hybridization with complementary DNA through electrical detection. Real-time current responses of noncomplementary and complementary DNA demonstrate a distinct difference. Significant current decrease suggests that the carrier depletion in InN film results from the attachment of negatively charged complementary DNA. These results show a great potential of using functionalized ultrathin InN ISFETs as biosensors for a wide variety sensing application.

ACKNOWLEDGMENTS

This work is partially supported by the National Science Council in Taiwan through the project Nos. NSC 98-3011-P-007-001 and NSC 97-3114-E-007-002.

REFERENCES

1. D. Gerion, F. Chen, B. Kannan, A. Fu, W. J. Parak, D. J. Chen, A. Majumdar, and A. P. Alivisatos, *Anal. Chem.* **75**, 4766 (2003).
2. A. W. Peterson, R. J. Heaton, and R. M. Georgiadis, *Nucleic Acids Res.* **29**, 5163 (2001).
3. X. Su, R. Robelek, Y. Wu, G. Wang, and W. Knoll, *Anal. Chem.* **76**, 489 (2004).
4. J. Fritz, M. K. Baller, H. P. Lang, H. Rothuizen, P. Vettiger, E. Meyer, H.-J. Güntherodt, Ch. Gerber, and J. K. Gimzewski, *Science* **288**, 316 (2000).
5. E. Souteyrand, J. Cloarec, J. Martin, C. Wilson, I. Lawrence, S. Mikkelsen, and M. Lawrence, *J. Phys. Chem. B* **101**, 2980 (1997).
6. Z. Li, Y. Chen, X. Li, T. Kamins, K. Nauka, and R. Williams, *Nano Letters* **4**, 245 (2004).
7. B. S. Kang, S. J. Pearton, J. J. Chen, F. Ren, J. W. Johnson, R. J. Therrien, P. Rajagopal, J. C. Roberts, E. L. Piner, and K. J. Linthicum, *Appl. Phys. Lett.* **89**, 122102 (2006).
8. Y.-S. Lu, C.-L. Ho, J. A. Yeh, H.-W. Lin, and S. Gwo, Appl. Phys. Lett. **92**, 212102 (2008).
9. C. M. Halliwell and A. E. G. Cass, *Anal. Chem.* **73**, 2476 (2001).
10. B. Kobrin, V. Fuentes, S. Dasaradhi, R. Yi, R. Nowak, J. Chinn, R. Ashurst, C. Carraro, and R. Maboudian, *Semiconductor Equipment and Materials International* (2004).
11. S. Gwo, C. L. Wu, C. H. Shen, W. H. Chang, T. M. Hsu, J. S. Wang, and J. T. Hsu, *Appl. Phys. Lett.* **84**, 3765 (2004).
12. B. Pattier, J.-F. Bardeau, M. Edely, A. Gibaud, and N. Delorme, *Langmuir* **24**, 821 (2008).