

ZnO Nanorod Biosensor for Highly Sensitive Detection of Specific Protein Binding

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We report on the fabrication of electrical biosensors based on functionalized ZnO nanorod surfaces with biotin for highly sensitive detection of biological molecules. Due to the clean interface and easy surface modification, the ZnO nanorod sensors can easily detect streptavidin binding down to a concentration of 25 nM, which is more sensitive than previously reported one-dimensional (1D) nanostructure electrical biosensors. In addition, the unique device structure with a micrometer-scale hole at the center of the ZnO nanorod's conducting channel reduces the leakage current from the aqueous solution, hence enhancing device sensitivity. Moreover, ZnO nanorod field-effect-transistor (FET) sensors may open up opportunities to create many other oxide nanorod electrical sensors for highly sensitive and selective real-time detection of a wide variety of biomolecules.

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I. INTRODUCTION

Nanosensors based on semiconductor nanostructures, such as carbon nanotubes (CNTs), nanowires, and nanorods, have recently attracted considerable attention for detecting biological molecules [1–5]. Among the variety of nanosensor systems, nanometer-scale electronic sensors based upon one-dimensional (1D) semiconductors offer high sensitivity and real-time detection [3,6,7]. For example, thanks to the high surface-to-volume ratio of the 1D nanostructures, the detection sensitivity of 1D field effect transistor (FET) biosensors may be increased to a single-molecular detection level by monitoring the very small change in conductance caused by binding of biomolecular species on a long conduction channel. 1D semiconductor electronic biosensors, in particular, have active surfaces that can easily be modified for immobilization of numerous biomolecules. However, this advantage may not apply to many non-oxide semiconductor nanomaterials because their surfaces are not stable in an air environment, which leads to formation of an insulating native oxide layer and may degrade device reliability and sensitivity [8]. FETs based on 1D oxide semiconductors may solve this problem [9–11]. Nevertheless, detection of biological molecules using 1D oxide semiconductor FETs has rarely been studied.

As one of the protein-receptor interactions, we selected a high-affinity biotin-streptavidin binding system

because this system is widely utilized in clinical diagnostic applications and has been under evaluation as a molecular component in tumor-targeted cancer therapeutics [12,13]. For the detection of protein-receptor interactions, we functionalized ZnO nanorod surfaces by using polyethylene glycol (PEG), similar to the case of carbon nanotube FETs [14]. Here, we report highly sensitive biological nanosensors based on air-stable, single-crystal ZnO nanorod FETs for electronic detection of specific protein binding.

II. EXPERIMENT

ZnO nanorod FETs with n-channel depletion modes (normally ON) were fabricated for real-time detection of biological species monitoring protein-receptor interactions. Prior to the fabrication of the ZnO nanorod FETs, high-quality, single-crystalline ZnO nanorods were prepared using catalyst-free metal-organic chemical vapor deposition [15,16], and then dispersed on silicon wafers with a 250-nm-thick thermal oxide. Source and drain electrode contacts were made by evaporating 80-nm-thick Ti and 100-nm-thick Au metal layers on ZnO nanorods, respectively. The details of the FET fabrication method are reported elsewhere [14]. Although high performance ZnO nanorod FETs were previously fabricated as reported elsewhere [17], they exhibited considerable leakage current passing through aqueous solution. Thus we further modified the biosensor structure

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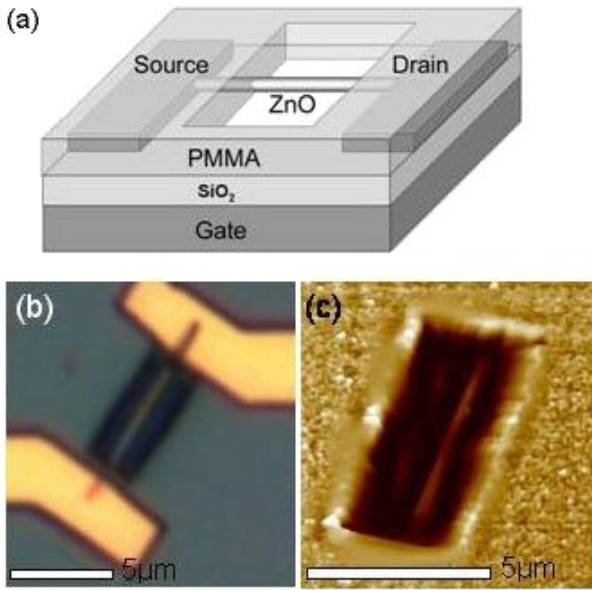


Fig. 1. (a) Schematic of single ZnO nanorod biosensor system and (b) optical microscopic and (c) AFM images. The biosensor has a micrometer-scale hole with a width of $2.5 \mu\text{m}$ and a length of $6 \mu\text{m}$ at the center of the ZnO nanorod's conducting channel.

by using a conventional e-beam lithography technique to make a micrometer-scale hole using a conventional e-beam lithography technique at the center of the ZnO nanorods conducting channel (Fig. 1(a)) in order to monitor the conductance of the nanorod itself in aqueous solution. Figs. 1(b) and (c) show optical microscopy and AFM images of the nanorod sensor, respectively, exhibiting a hole with a width of $2.5 \mu\text{m}$ and a length of $6 \mu\text{m}$. In this nanosensor structure, the source and the drain contact electrodes are buried in copolymer/poly methyl methacrylate (PMMA) with a thickness of $2 \mu\text{m}$, significantly reducing the leakage current through the aqueous solution and enhancing the sensitivity and the reliability of the nanorod biosensor.

ZnO nanorod FETs detect biological or chemical molecules by measuring changes in conductance. ZnO nanorod surfaces have been modified by coating them with PEG or applying one drop of biotin solution (0.17 M solution of 10 mg biotin-PEG wax in de-ionized water) onto a nanodevice, followed by the transport measurements. The biotin-modified ZnO nanorod devices were then exposed to 0.025 -, 0.25 -, and 2.5 - μM solutions of streptavidin in 0.01 -M phosphate buffered saline ($\text{PH} = 7.2$) in sequence by monitoring the real time conductance change. After each exposure, the source-drain current-voltage (I_{ds} - V_{ds}) characteristics were measured after the conductance had been stabilized. For the gate bias dependent current-voltage measurements, V_g was swept from a negative bias to a positive bias and then back to a negative bias with a sweep rate of 0.2 V/s . All electrical measurements were performed at room temper-

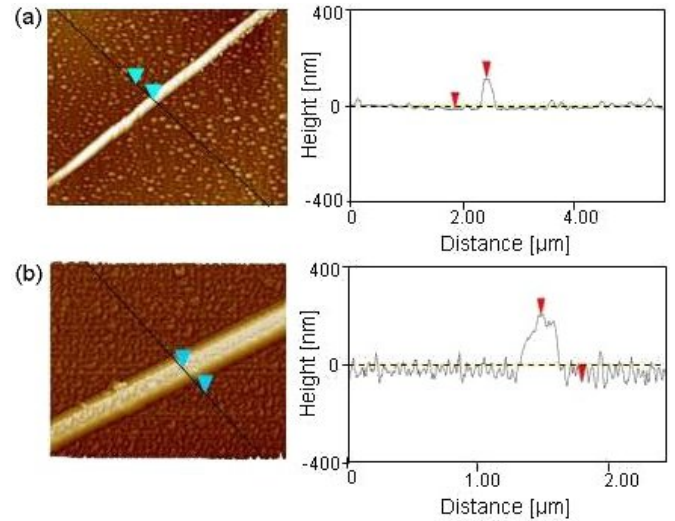


Fig. 2. AFM topographic images of a biotin-modified ZnO nanorod (a) before and (b) after exposure to streptavidin labeled with gold nanoparticles. After the exposure, bright dots with diameters of 10 nm are clearly observed, and the nanorod diameter shown as the height in the line profiles increased from $120 - 130 \text{ nm}$ to 270 nm , indicating that streptavidin is attached to biotin-modified ZnO nanorods.

ature under an ambient air environment.

III. RESULTS AND DISCUSSION

Atomic force microscopy (AFM) was employed prior to electrical measurements of the nanorod biosensors in order to investigate changes in the nanorod surface morphology after functionalization with biotin and subsequent exposure to streptavidin labeled with gold nanoparticles. Bare ZnO nanorods displayed a clean ZnO surface, but the AFM image of ZnO nanorod after biotin-modification in Fig. 2(a) exhibits a bumpy surface due to biotin molecules coated on the ZnO nanorod surface. In the image of the ZnO nanorods exposed to streptavidin labeled with gold nanoparticles with a 10 nm diameter, however, bright dots with diameters of 10 nm are clearly observed, indicating that streptavidin is effectively attached to biotin-modified ZnO nanorods. The biotin-streptavidin binding on the ZnO surface is also confirmed by the increase in nanorod diameter (height in the AFM images). As shown in the line profiles crossing a ZnO nanorod (Fig. 2), the ZnO nanorod diameter increased from $120 \sim 130 \text{ nm}$ to 270 nm after streptavidin attachment on the biotin-modified nanorod.

ZnO exhibits strong adsorption of molecules on the surface, which affects the electrical characteristics of ZnO-based devices, dependent on surface-mediated phenomena. For example, the conductance of ZnO nanorod FETs drastically changes when molecules adsorb on the ZnO surface. Fig. 3(a) shows the source-drain current-

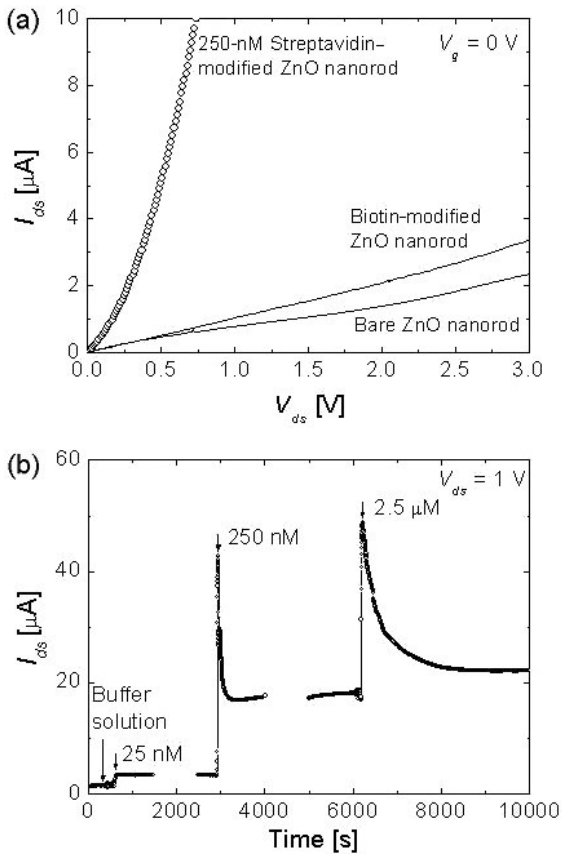


Fig. 3. (a) I_{ds} - V_{ds} curves of bare, biotin-modified, and streptavidin-exposed ZnO nanorod FET. No gate bias was applied during these measurements. (b) Current versus time for the biotin-modified ZnO nanorod device following addition of 0.025, 0.25, and 2.5 μM streptavidin, respectively. The gate and the source-drain voltages were 0 and 1 V, respectively.

voltage (I_{ds} - V_{ds}) characteristic curves of a ZnO nanorod FET before and after functionalization of ZnO nanorod surfaces with biotin, and binding of streptavidin with the biotin-functionalized surface. The ZnO nanorod FET exhibited only a small increase from 0.77 to 1.0 μS in conductance even after the biotin-functionalization, at a source-drain voltage (V_{ds}) of 1 V and a gate voltage (V_g) of 0 V. However, after the biotin-modified ZnO nanorod FET had been exposed to a 250 nM streptavidin solution, the conductance at the same voltages drastically increased to 17 μS , presumably due to the biotin-streptavidin binding. The order of magnitude change in the conductance of the ZnO nanorod FET upon exposure to streptavidin solution is significantly higher than the conductance change of 3 % from 1600 to 1650 nS after the exposure of Si nanowire FET sensors to a 250-nM streptavidin solution [3].

Additional control experiments were performed in order to confirm that the conductance change resulted from the specific binding of streptavidin to the biotin ligand on the ZnO nanorod's surface. Most significantly, when

bare ZnO nanorod devices (without biotin modification) were exposed a 250-nM streptavidin solution, the conductance change was less than a few percent, representing a weak electrical interaction or charge transfer between the bare ZnO nanorod surface and streptavidin. As in Fig. 3 shown, additionally, the ZnO nanorod biosensors with biotin-modified surfaces did not show any significant changes in conductance due to exposure to a buffer solution without any streptavidin. However, the conductance of the ZnO nanorod FET with a biotin-modified surface responded significantly to a streptavidin solution with even a small concentration of 25 nM. These results strongly suggest that the conductance change in the biosensor results from the biotin-streptavidin binding.

Fig. 3(b) shows plots of current versus time for a biotin-modified ZnO nanorod FET following sequential additions of buffer solutions without streptavidin and with 0.025-, 0.25-, and 2.5- μM streptavidin, where V_g and V_{ds} were fixed at 0 and 1 V, respectively. The arrows indicate conductance changes observed whenever a solution with streptavidin was added. Even with a 25-nM streptavidin solution, the conductance change was as large as 1.8 μA , corresponding to an increase of 140 % in conductance. In addition, device conductance increased almost linearly with increasing streptavidin concentration up to 250-nM. However, further addition of 2.5- μM streptavidin resulted in only a small change in device conductance. Since most biotin-binding sites are bonded with streptavidin molecules after exposure to the 250-nM solution, only a small number of empty biotin-binding sites remains, which presumably results in only a small change in the conductance at high streptavidin concentrations above 250-nM. These electronic sensing experiments indicate that a rapid and drastic conductance change is observed even for small streptavidin concentration. Addition of streptavidin solution, produced drastic conductance changes in a few seconds, and the highest conductance in was reached 25 ~ 30 seconds. Moreover, the conductance decayed exponentially after reaching the peak. Accordingly, we strongly suggest that ZnO nanorod FET sensors can be used for highly sensitive and specific real-time molecular recognition.

The changes in the electrical characteristics resulting from the biotin-streptavidin interaction were further investigated by measuring the I_{ds} - V_g characteristics of ZnO nanorod devices. Figs. 4(a) and (b) show the I_{ds} - V_g curves of ZnO nanorod FETs without and with biotin-streptavidin complexes, respectively. Streptavidin binding resulted in an increase in the conductance response to V_g or transconductance ($g_m = dI_{ds}/dV_g$), accompanied by an increase in the maximum ON state current and a decrease in the absolute value of the threshold voltage. That is, from the I_{ds} - V_g curve sweeps in a forward direction from negative to positive bias, g_m increased from 0.3 to 4 μS , the maximum ON state current increased from 3 to 20 μA , and the threshold voltage increased from -30 to -5 V. Meanwhile, a large hysteresis curve was

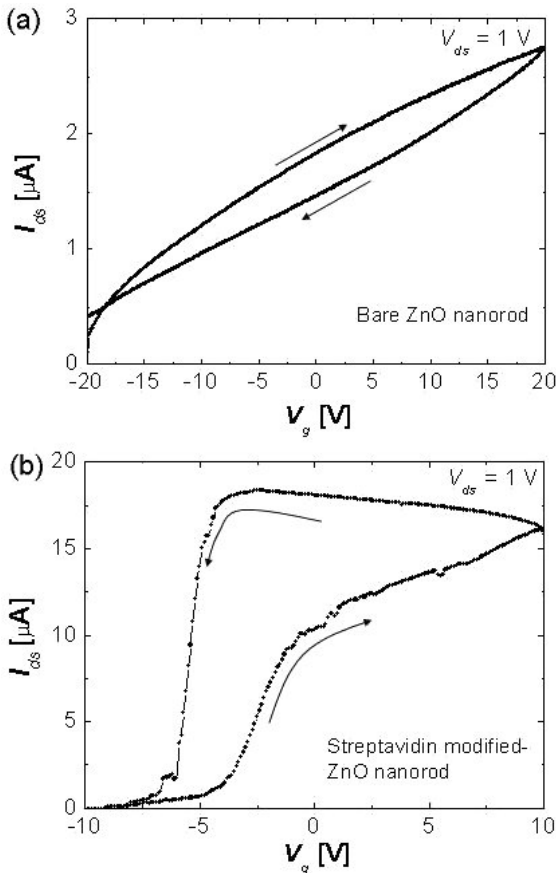


Fig. 4. I_{ds} - V_g curves of the ZnO nanorod FETs (a) before and (b) after biotin-streptavidin binding. The source-drain voltage (V_{ds}) was 1 V.

also observed, which was attributed to adsorbed molecular species working as charge traps [18]. The hysteresis effect has previously been observed in most instances of ligand-receptor interactions, as well as in simple adsorption of proteins onto solid surfaces [19,20].

The observed changes in the conductance and in the current-voltage characteristics due to biotin-streptavidin binding may be explained in terms of a chemical gating effect and a charge transfer process [3,9,21]. We believe that these different processes coexist in ZnO nanorod biosensors. That is, the conductance reduction for negative gate voltages ($V_g < \sim -5$ V) and threshold voltage shifts presumably result from the chemical gating effect [3,9]. In an n-channel ZnO nanorod FET, this chemical gating effect from the binding of the negatively charged streptavidin induces electron depletion, thereby rendering the device less n-type. For positive gate voltages, however, the conductance increases presumably due to charge transfer from streptavidin to the n-type ZnO channel via biotin [21]. The charge transfer process may also result in a drastic increase and subsequent slow decrease in the FET conductance right after device exposure to the streptavidin solution, as indicated by

the arrows in Fig. 3(b). That is, as soon as the streptavidin binds to biotin on a ZnO nanorod FET, excess electron carriers are donated from charged streptavidin to ZnO, presumably resulting from the absence of an insulating barrier layer between the ZnO and the biotin-streptavidin complex.

IV. CONCLUSIONS

We fabricated electrical biosensors based on functionalized ZnO nanorod surfaces with biotin for highly sensitive detection of biological molecules. The ZnO nanorod sensors can easily detect streptavidin binding down to a concentration of 25-nM, which is more sensitive than previously reported 1D electrical biosensors, including Si nanowire biosensors and carbon nanotube FET sensors. Presumably this may result from the clean interface between the ZnO nanorod surface and the biological or chemical species and from easy surface modification of oxide surfaces for immobilization of the species. In addition, our unique FET device structure with a micrometer-scale hole at the center of the ZnO nanorod's conducting channel reduces the leakage current from the aqueous solution, hence enhancing device sensitivity. More generally, we believe that ZnO nanorod electrical sensors may be expanded to create many other oxide nanorod electrical sensors for highly sensitive and selective real-time detection of a wide variety of biomolecules.

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