Electronic Detection of Specific Protein Binding Using Nanotube FET Devices

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ABSTRACT

We have used nanoscale field effect transistor devices with carbon nanotubes as the conducting channel to detect protein binding. A PEI/PEG polymer coating layer has been employed to avoid nonspecific binding, with attachment of biotin to the layer for specific molecular recognition. Biotin-streptavidin binding has been detected by changes in the device characteristic. Nonspecific binding was observed in devices without the polymer coating, while no binding was found for polymer-coated but not biotinylated devices. Streptavidin, in which the biotin-binding sites were blocked by reaction with excess biotin, produced essentially no change in device characteristic of the biotinylated polymer-coated devices.

Current biological sensing techniques commonly rely on optical detection principles that are inherently complex, requiring multiple steps between the actual engagement of the analyte and the generation of a signal, multiple reagents, preparative steps, signal amplification, complex data analysis, and relatively large sample size. The techniques are highly sensitive and specific but more difficult to miniaturize. Electronic detection techniques may offer an alternative, but their potential has not yet been explored fully. Field effect transistors (FETs) fabricated using semiconducting single wall carbon nanotubes (nanotube FETs, NTFETs) have been extensively studied.[1,2] Such devices have been found to be sensitive to various gases, such as oxygen and ammonia, and thus can operate as sensitive chemical sensors. The mechanism responsible for the change of device characteristic is thought[3] to be a charge-transfer reaction between the analytes and the nanotube. NTFET devices,[3,4] together with devices based on nanowires,[5] are also promising candidates for electronic detection of biological species. Various groups have examined the conformational compatibility−driven by size issues as well as hydrophobic effects−between proteins and carbon nanotubes using streptavidin, and found that the protein is able to crystallize in a helical conformation around multiwall carbon nanotubes.[6] We have also shown[7] that functionalization of the nanotubes with carboxylic groups, thereby rendering them more hydrophilic, does not lead to protein attachment, thus opening up the avenues for specificity. Researchers have also made[3c,4] some attempts at functionalizing single-wall carbon nanotubes to make them biocompatibile, capable of recognizing proteins by using noncovalent binding between a bifunctional molecule and the nanotube to anchor a bioreceptor molecule with a high degree of control and specificity.

In this communication we report taking these advances one step further, by using a sensor architecture that allows the detection of protein−receptor interactions (using biotin−streptavidin binding as an example) and, at the same time, reduces or eliminates nonspecific protein binding. Figure 1 schematically depicts a sensor architecture that uses a NTFET as a transducer; it is covered with a polymer coating that has hydrophilic properties and onto which biotin is attached. Polymer functionalization in this sensor architecture has several advantages. First, the polymer is used to attach molecular receptor molecules to the sidewalls of nanotubes. Several examples of covalent chemical attachment of biological molecules to nanotubes, including proteins and DNA, have been recently published.[8] Covalent modification, however, has the disadvantage that it impairs physical properties of carbon nanotubes. For these reasons we have employed a supramolecular approach, namely, the noncovalent functionalization of carbon nanotubes by employing polymer coatings.[9] Second, polymer coatings have been shown to modify the characteristics of nanotube FET devices, and thus the coating process can be readily monitored. In particular, coating NTFETs with polyethylene imine (PEI) polymer was found[10] to shift the device characteristic from p- to n-type, presumably due to the electron-donating ability of amine groups in the polymer. Third, the polymer coating could be used to prevent nonspecific binding of proteins. A variety of polymer coatings and self-assembled monolayers have been used to prevent binding of undesired species on surfaces for biosensor and biomedical device applications.[11] Among the various available polymers for coating, poly(ethylene

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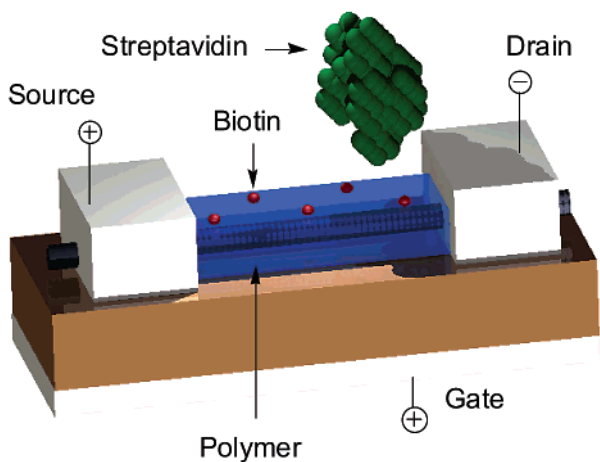† On leave from Department of Physics, University of California, Los Angeles.

Figure 1. Schematic of the nanotube field effect transistor (NTFET). A polymeric functional layer, which coats the nanotube, is functionalized with a molecular receptor, biotin, a protein that recognizes a biomolecule, streptavidin. (Dimensions are not in scale.)

Figure 2. Biotinylation reaction of the polymer layer (PEI and PEG).

glycol) (PEG) is one of the most effective and widely used. This layer, due to its hydrophilicity, reduces the affinity of nanotubes toward protein binding.

We have chosen the biotin−streptavidin binding to demonstrate the effectiveness of the device architecture. This binding serves as a model system for protein interactions,[12] has been extensively studied, and the binding is well understood. In our procedure, after incubation, the device was washed and dried, and the device characteristics were examined after drying. While we have explored the device response in a buffer, our objective here is to examine the changes of the device characteristic, brought about by the different chemical and biological modifications on the electronic response, such direct correspondence being somewhat obscured in a buffer environment.[13]

FET devices with nanotubes as the conducting channel were fabricated using nanotubes grown by chemical vapor deposition (CVD) on 200 nm of silicon dioxide on doped silicon from iron nanoparticles with methane/hydrogen gas mixture at 900 °C; electrical leads were patterned on top of the nanotubes from titanium films 35 nm thick capped with gold layers 5 nm thick, with a gap of 0.75 μm between source and drain. Multiple nanotubes connected the source and drain electrodes, with the individual tubes varying from metallic to semiconducting.[14] Consequently, a range of device modulations (expressed as the ratio of the "on" to the "off" source−drain current, measured at −10 V and +10 V gate voltage, respectively) were observed. The devices displayed p-type transistor behavior, as has also been observed by others.[1,2] In this paper we have examined the dependence of the source−drain current, I_{sd}, as function of the gate voltage V_g, $I_{sd}(V_g)$, measured from +10 V to −10 V, and we refer to this response as the "device characteristic". After conducting initial electrical measurements to establish the device characteristic, the substrates were submerged in a 10 wt % solution of poly(ethylene imine) (PEI, average molecular weight ~25 000, Aldrich) and poly(ethylene glycol) (PEG, average molecular weight 10 000, Aldrich) in water over-
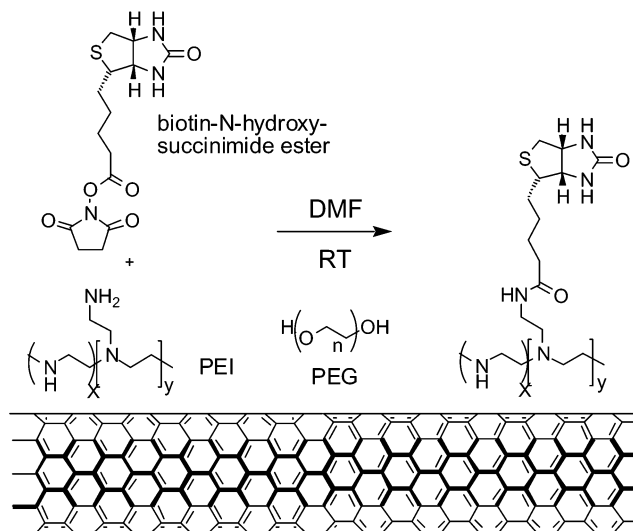
night, followed by thorough rinsing with water. Commercial polyethyleneimine (PEI) was used; this form is highly branched, has a molecular weight of about 25 000, and contains about 500 monomer residues. About 25% of the amino groups of PEI are primary with about 50% secondary, and 25% tertiary. A thin layer (<10 nm) of polymer material coated the devices, as observed by atomic force microscopy. The polymer-coated devices were biotinylated by submerging them in a 15 mM DMF solution of biotin-*N*-hydroxysuccinimide ester (Sigma) at room temperature. This compound readily reacts with primary amines in PEI under ambient conditions, leading to changes of the device characteristic as will be discussed below. After soaking overnight, devices were removed, rinsed with DMF and deionized water, blown dry in nitrogen flow, and dried in a vacuum. Figure 2 depicts the scheme by which biotin was attached to the polymer coating. The biotinylated polymer-coated devices were exposed to the 2.5 μM solution of streptavidin[15] in 0.01 M phosphate buffered saline (pH = 7.2, Sigma) at room temperature for 15 min. Subsequently, the devices were thoroughly rinsed with deionized water and blown dry with nitrogen. Several control experiments have also been performed in order to demonstrate the effectiveness of the polymer layer in the prevention of nonspecific binding.

An atomic force microscope (AFM) image of one of the devices after exposure to streptavidin labeled with gold nanoparticles is shown in Figure 3. Light dots represent gold nanoparticles (10 nm), and thus indicate the presence of streptavidin. Based on the image, we conclude that streptavidin is effectively attached to the nanotubes, due to the strong adsorption of the PEI polymer to the sidewalls of the nanotubes, which was biotinylated after deposition. With a nanotube length of 800 nm and a gold sphere diameter of 10 nm, it is expected that, upon full coating, there are approximately 80 streptavidin molecules in direct interaction with the nanotube conducting channel. (This assumes that, on the average, one streptavidin molecule per gold nanoparticle is attached to the nanotube.)
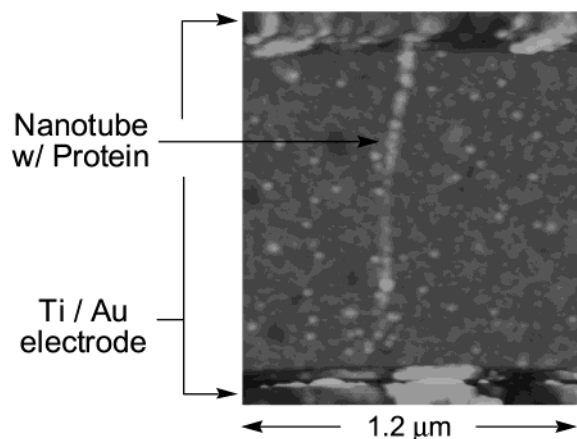
Figure 3. AFM image of the polymer-coated and biotinylated NTFET after exposure to streptavidin labeled with gold nanoparticles (10 nm diameter).
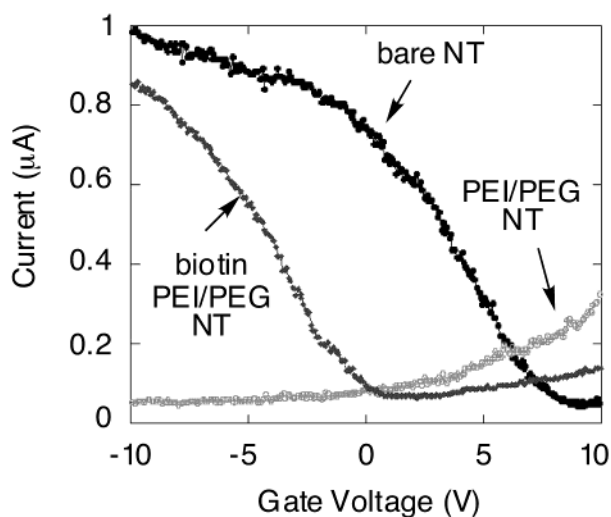

Figure 4. Gate voltage dependence of the source-drain current I_{sd} of a typical device before and after PEI/PEG polymer coating and after biotin attachment to the polymer layer.

Next we discuss the change of the device characteristic in response to the steps we have taken. The device characteristic before chemical modification is p-type, in an ambient environment, presumably due to exposure to oxygen.[16] Coating the device with the mixture of PEI and PEG polymers results in an n-type device characteristic (Figure 4). This effect, which has been observed[10] before, probably results from the electron donating property of the NH_2 groups of the polymer. The electronic characteristic of the device after 18 h of biotinylation reaction is also depicted in Figure 4. Attachment of biotin is through covalent binding to the primary NH_2 group, thereby reducing the overall electron donating function of PEI and leading to a device characteristic that is consistent with removal of electrons from the device. As only the primary NH_2 sites are involved in binding to biotin, the p-type conductance observed before coating is not fully recovered.[17]

The effect of exposing the biotinylated polymer-coated device to a streptavidin solution and the control experiments (conducted on different devices) are shown in Figure 5.

Figure 5a shows a striking loss of source−drain current for negative gate voltages after exposure to streptavidin and consequent streptavidin−biotin binding with little evidence for the shift of the device characteristic toward negative or positive gate voltage. Several control experiments were performed to demonstrate the effectiveness of the device architecture in avoiding false positives and in detecting specific protein binding. First, we have exposed the uncoated NTFET device to streptavidin and have found a change of the device characteristic, as shown in Figure 5b, indicating attachment of streptavidin to the device. Note, however, that in this case the primary effect is the shift of the device characteristic toward negative gate voltage. In contrast, when the device was polymer-coated, but not biotinylated, no changes occurred upon exposure to streptavidin (Figure 5c). This suggests the effectiveness of the polymer coating in preventing direct, nonspecific interaction of streptavidin with the nanotube. Finally, addition of a streptavidin in which the biotin-binding sites were blocked by complexation with excess biotin produced essentially no change in device characteristic of the biotinylated polymer-coated device (Figure 5d).

Several conclusions on the effect of biomolecules on the device electronics can be drawn. First, exposing the bare, uncoated device to streptavidin leads to the shift of the transconductance toward negative gate voltages, thereby rendering the device less p-type, with little reduction in the magnitude of the transconductance. This indicates that the primary effect of the nanotube-streptavidin binding is a charge-transfer reaction with streptavidin donating electrons to the nanotube.[16] Biotin−streptavidin binding has a different effect; in this case the I_{sd} is reduced. We suggest that upon streptavidin−biotin binding, geometric deformations occur, leading to scattering sites on the nanotube, and thus to reduced conductance. At the same time the device characteristic is modified only for negative gate voltages (see Figure 5a), leaving the transconductance in the positive gate voltage region unaffected. We have observed[17] similar features in devices to which charge carriers were deposited, and we have argued that the observation is due to localization (delocalization) of positively (negatively) charged ionic entities by a negatively (positively) charged surface. Such a mechanism may also be effective here, and the mechanism may open the way for electronic modification of bioreactions.

With improvements in NTFET devices, they may also be rendered sensitive enough that single protein detection and monitoring can be achieved. As can be inferred from Figure 5a, the total change in transconductance exceeds the noise level by a factor of 10. According to an AFM image of the device (Figure 3), there are about 100 gold nanoparticles, and approximately 100 protein molecules (assuming one protein per gold nanoparticle binding to the tube) in close proximity to the carbon nanotube. Combining these two numbers, our current detection level can be estimated to be of the order of 10 streptavidin molecules. Similar detection sensitivity can be inferred from experiments we have conducted on uncoated nanotubes incubated with streptavidin (Figure 5b). This is in contrast to the relatively modest
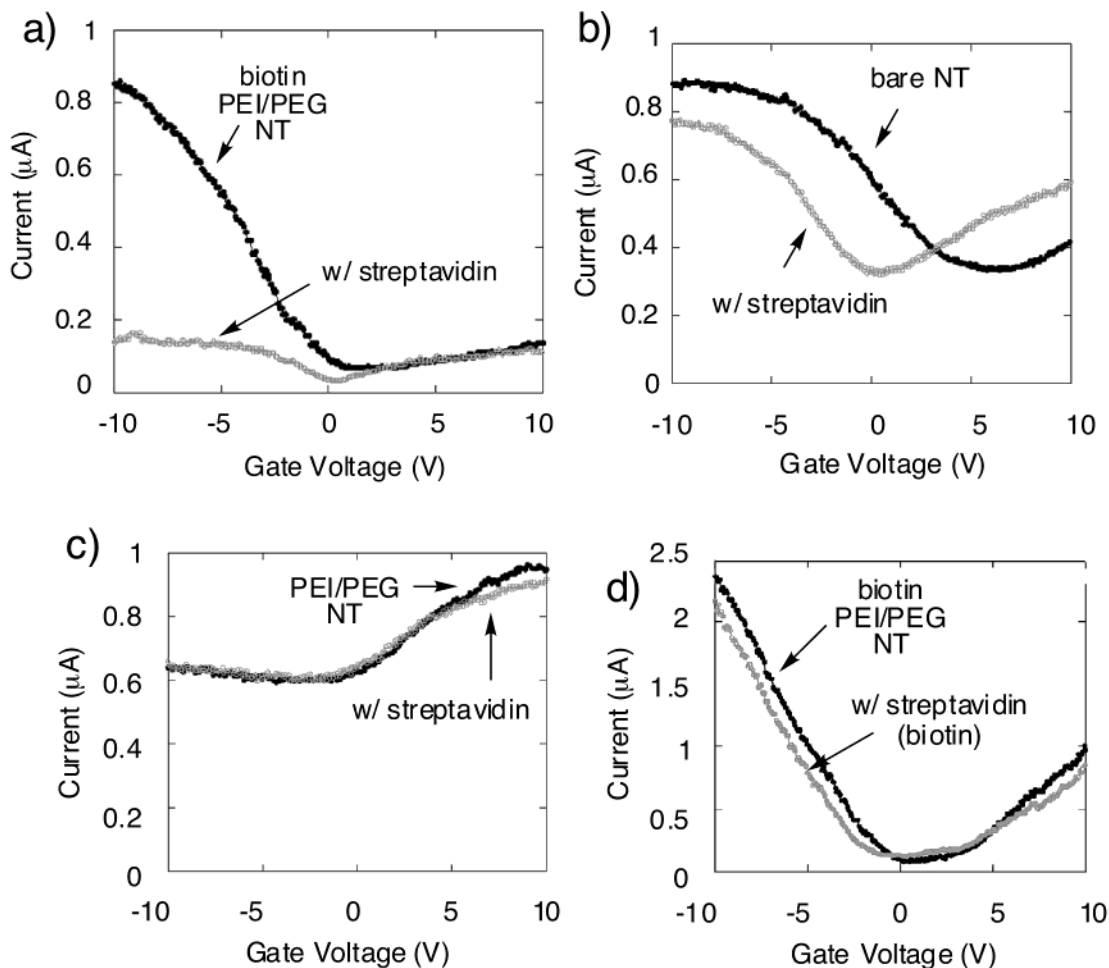
Figure 5. Change of the device characteristic $I_{sd}(V_g)$ upon exposure to streptavidin. Different devices with similar characteristic as observed before polymer coating were used for a, b, and c. (a) Gate voltage dependence I_{sd} of the biotinylated, polymer-coated NTFET in the absence and in the presence of streptavidin. (b) Current−voltage dependence $I_{sd}(V_g)$ of the bare NTFET device to nonspecific protein binding. (c) $I_{sd}(V_g)$ of the polymer-coated NTFET device in the absence and presence of streptavidin. (d) $I_{sd}(V_g)$ of the biotinylated, polymer-coated NTFET device in the absence and presence of streptavidin that was preincubated with biotin.

change observed in devices where the active element is a nanowire[5] − a channel with a substantially larger cross section.

Electronic sensing using devices with nanotubes as the conducting channel offers several advantages. Such sensors are small and fast, and the active detection area is sized for individual proteins or viruses. These sensors are extremely sensitive, as all the current passes through the detection point. Most importantly, at a later stage the devices can be made specific to individual molecules; potentially their response to different species can be varied in a controlled way using chemical and biological functionalization.

These concepts could conceivably be extended at a later stage to include cell-based electronic sensing (measuring the electronic response of living systems) and to using nanoscale devices for in-vivo applications (studying cell physiology, medical screening and diagnosis). The devices can be turned into devices where, by applying a voltage between elements of the sensor, surface charges can be created on the sensing element where the bio-molecules are immobilized. Such surface charges will interact with the charged biomolecules affecting biological function. Our experiments on that aspect

of the electronic device−biology interface will be reported in a subsequent publication.

Supporting Information Available: An AFM image of a typical semiconducting nanotube device is presented in Supporting Information. The device characteristic at different stages of the biotinylation reaction is presented. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) NTFETs were fabricated using nanotubes grown by chemical vapor deposition, directly on 4" silicon wafers (with 200 nm films of thermal SiO_2) using a home-built apparatus. In a typical experiment, the wafer is covered with patterned photoresist and is spin coated with growth promoter containing nanoparticles of iron encased within a mesoporous material [(a) Li, W. Z.; Xie, S. S.; Qian, L. X.; Chang, B. H.; Zou, B. S.; Zhou, W. Y.; Zhao, R. A.; Wang G. *Science* **1996**, *274*, 1701−1703, (b) Pan, Z. W.; Xie, S. S.; Chang, B. H.; Wang, C. Y.; Lu, L.; Liu, W.; Zhou, W. Y.; Li, W. Z.; Qian, L. X. *Nature* **1998**, *394*, 631−632]. After liftoff in acetone, small patterned areas of the growth promoter are left on the wafer. The wafer is then introduced in a 5 in. tubular oven and treated at 900 °C in a methane and hydrogen flow for 15 min, allowing for the growth of SWCNT of 5−10 μm long and 1.5 to 3 nm in diameter. The hydrogen helps to prevent the deposition of amorphous carbon around the nanotubes as well as on the surface of the silicon [Ivanov V.; Nagy, J. B.; Lambin, Ph.; Lucas, A.; Zhang, X. B.; Zhang, X. F.; Bernaerts, D.; Van Tendeloo, G.; Amelinckx, S.; Van Landuyt, J. *Chem. Phys. Lett.* **1994**, *223*, 329−335]. Standard optical lithography and metal deposition are used to form the metal contacts on top of the grown nanotubes. A fair proportion of the tens of thousands of devices made on each wafer are p-type FETs with a modulation of 1, indicating that only semiconducting nanotubes are present. The devices used in this study were selected from among those. An AFM image of a typical device is presented in Supporting Information.

(15) Streptavidin is labeled with gold nanoparticles for the purpose of AFM imaging. Streptavidin (from *Streptomyces avidinii*, Sigma Chemicals) without gold labeling had similar effect on the device characteristic but could not be detected by AFM.

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(17) The progress of the on-chip biotinylation reaction can be monitored by measuring $I_{sd}(V_g)$. Biotin-*N*-hydroxysuccinimide ester reacts readily with primary amines in PEI under ambient conditions, thus reducing the electron donating of PEI. However, after 1 h the yield of the reaction is only ∼75%; several hours are required to complete the reaction. The device characteristic at different stages of the biotinylation reaction is presented in Supporting Information.

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