Communication

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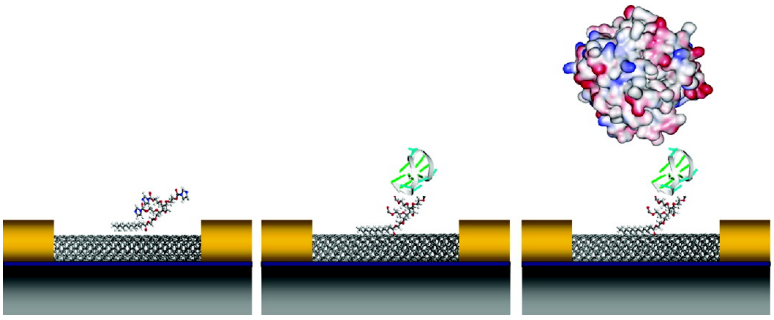

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Single-Walled Carbon Nanotube Biosensors Using Aptamers as Molecular Recognition Elements

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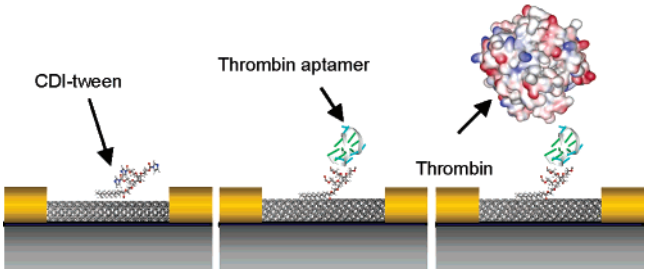
One of the biggest successes in nanobiotechnology relates to the development of the next generation biological sensors. Recently, one-dimensional nanostructures, such as carbon nanotubes and semiconductor nanowires, have been successfully demonstrated as sensitive biological sensors. It has been reported that the real-time detection of single viruses,[1] small molecules,[2−4] and proteins[5−7] is possible with biosensors that use nanowire or carbon nanotube transistors as the active transducer. In previous research, proteins, such as enzymes and antibodies, that are specific to each target have been used as probes. Proteins as recognition receptors are highly advantageous, as they are highly specific, yet disadvantageous because they are relatively unstable and production costs for antibodies are rather high. Instability of recognition elements greatly reduces the shelf life of a biosensor and, as such, is a major concern that needs to be addressed if commercialization of these nanoscale devices is to be realized.

Aptamers are artificial oligonucleotides (DNA or RNA) that can bind to a wide variety of entities (e.g., metal ions, small organic molecules, proteins, and cells) with high selectivity, specificity, and affinity, equal to or often superior to those of antibodies. These aptamers can be isolated from combinatorial nucleic acid libraries using in vitro selection methods.[8−10] Synthesizing aptamers is relatively inexpensive, and they can be engineered easily for immobilization purposes. Moreover, unlike proteins, which are irreversibly denatured in unfavorable conditions, aptamers are capable of reversible denaturation. Consequently, by incorporating these aptamers into biosensors, it is possible to subject these sensing elements to repeated use, thereby realizing a device that is potentially recyclable.

Here, we report the first successful demonstration of a single-walled carbon nanotube field effect transistor (SWNT-FET) biosensor using aptamers as an alternative to protein-based sensing elements. Although peptide nucleic acid (PNA) has been used as the recognition element in certain Si nanowire biosensors, PNA is quite different from aptamers in that it can detect only nucleic acids through PNA−DNA hybridization.[11]

The biggest merit of using DNA (RNA) aptamers in FET-type sensors lies in their small size. In the case of immunological field effect transistors (ImmunoFETs), which use an antibody−antigen binding recognition step, there is a high possibility that the recognition binding occurs outside the electrical double layer in physiological salt concentrations.[12] In this respect, the antibody (∼10 nm) is much larger than the electrical double layer, such that most of the protein charges will be at a distance greater than the Debye length (∼3 nm in 10 mM ionic concentrations), making them impossible to detect. Since aptamers (1−2 nm) are much smaller than protein antibodies, it is possible that the aptamer−protein binding event can occur inside the electrical double layer in millimolar salt concentrations.

Scheme 1. Binding of Thrombin on a SWNT-FET-Based Aptamer Sensor



A 15-mer single-stranded DNA aptamer that binds to the blood-clotting factor, thrombin, was chosen as a model system. Recently, thrombin aptamers have successfully been demonstrated as molecular recognition receptors using various transducers.[13−15] We have immobilized thrombin aptamers (5′-GGTTGGTGTGGTTGG-3′, Bioneer Inc.) onto the side wall of a carbon nanotube transistor, pretreated with carbodiimidazole-activated Tween 20 (CDI-Tween).[7] For the covalent binding of thrombin aptamers to CDI-Tween, the 3′-end of the thrombin aptamer was modified with $-NH_2$ groups (see Supporting Information). In Scheme 1, we show a diagram of the thrombin binding on thrombin aptamer immobilized SWNT-FETs.

For our experiment, the SWNT-FETs were prepared using standard chemical vapor deposition technique, and detailed sample fabrication procedures can be found in Supporting Information. Aptamer immobilization was performed by first modifying the side wall of the carbon nanotube with CDI-Tween. While the Tween component was bound to the carbon nanotube side wall through hydrophobic interactions, the carbodiimidazole moiety was used to covalently attach the 3′-amine group of the thrombin aptamer. Then, the devices were allowed to react with a (100 pM) thrombin aptamer solution overnight. The electrical transfer characteristics of the SWNT-FET were measured at each process stage (see Supporting Information). The immobilization of the thrombin aptamer caused a rightward shift in the gate-threshold voltage, presumably due to the negatively charged DNA backbone. This shift, together with a small concomitant decrease in the conductance, was observed in all of the devices functionalized with DNA aptamers.

We conducted an experiment to measure the effect of thrombin on an SWNT-FET functionalized with a thrombin aptamer. Figure 1a shows the real-time measurement of conductance from the thrombin aptamer modified SWNT-FET. To minimize the environmental effect, a tungsten probe tip was used as a reference electrode. First, a 5 μL droplet of DI water was placed on the

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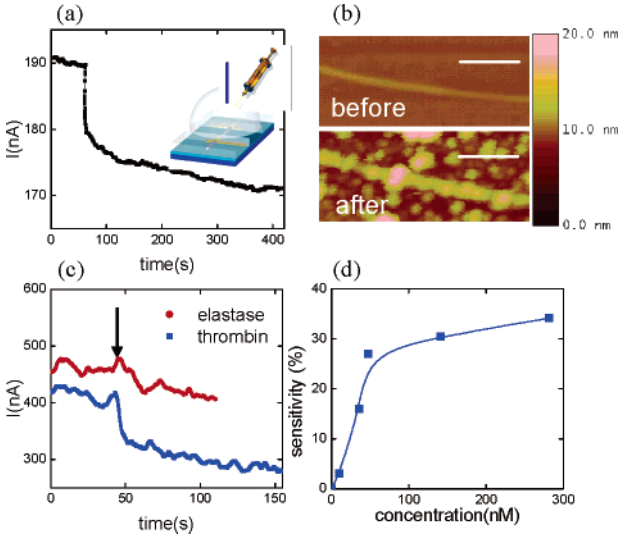

Figure 1. (a) A real-time conductance measurement obtained from the thrombin aptamer immobilized SWNT-FET. Inset shows a schematic diagram of the experimental setup. (b) AFM images of a SWNT before and after the thrombin binding experiment. Scales bars: 100 nm. (c) The selectivity of thrombin aptamer immobilized SWNT-FET. Arrow indicates the point of adding protein solution. (d) The sensitivity of SWNT-FET aptamer sensor as a function of thrombin concentration.

aptamer-modified SWNT-FET. As shown in Figure 1a, the conductance dropped sharply as soon as a 1.5 μmol thrombin solution was added to the DI water droplet. After the initial abrupt decrease, the conductance decreased more slowly until it reached the saturation point. As shown in the AFM image of Figure 1b, the whole aptamer SWNT-FET surface is completely covered by protein. The height of the globular protein measured by dry tapping mode AFM is around 3 nm. As a control, we conducted the same experiment using a SWNT-FET with no thrombin aptamers attached. In this case, no measurable change in conductance was observed (data not shown). The sudden drop in conductance observed above could be explained either by the thrombin molecules screening the negative charges of the DNA aptamer when the complex is formed or by the fact that the thrombin molecules will be positively charged under these experimental conditions (pH 5.4) since the isoelectric point of thrombin is rather high (7.0−7.6).[16]

To measure the selectivity of thrombin aptamers, we used a different protein, elastase. Elastase is another serine protease with an isoelectric point and molecular weight similar to that of thrombin. As shown in Figure 1c, the addition of elastase to the DI water droplet did not affect the conductance of the thrombin aptamer functionalized SWNT-FET (red curve). Following removal of the elastase solution, the sample was rinsed with clean DI water and then reacted with the thrombin solution. Again, adding thrombin to the thrombin aptamer functionalized SWNT-FET surface caused a sharp decrease in conductance (blue curve in Figure 1c), thereby demonstrating the selectivity of the immobilized thrombin aptamers.

Finally, we measured the sensitivity of the SWNT-FET-based aptamer sensors using varying protein concentrations. The same thrombin aptamer immobilized SWNT-FET was used for all measurements, and the sensor was "reset" by washing with 6 M guanidine hydrochloride solution, thereby removing the already bound thrombin molecules. Figure 1d shows the sensitivity ($\Delta I/I_0 \times 100$) of the SWNT-FET aptamer sensor as a function of thrombin concentration. As shown in Figure 1d, the sensitivity becomes saturated around protein concentrations of 300 nM, where the linear response regime of the sensor is expected to occur within the 0−100 nM range. Although the LOD (lowest detection limit) of the sensor used in this work is around 10 nM, we believe that it is possible to improve this sensitivity up to the subnanomolar level by using high-quality SWNT-FETs.[17] Extremely sensitive aptamer-based FET biosensors, using high performance SWNT-FETs (on/off ratio of 10^4), are currently being developed in our lab.

In conclusion, we have successfully demonstrated the first SWNT-FET-based biosensor comprising DNA aptamers as the molecular recognition elements. The fast response, high sensitivity, and relatively simple fabrication of these SWNT-FET sensors, combined with the small size, economy, stability, and high selectivity of aptamers, could provide a cost-effective point-of-care testing tool and a new method for high-throughput screening, as well.

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Supporting Information Available: Three-dimensional structures of a thrombin aptamer and a thrombin−thrombin aptamer complex; the experimental protocols and evolution data for the electrical transfer characteristics of SWNT-FETs. This material is available free of charge via the Internet at http://pubs.acs.org.

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