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Biotin-Streptavidin Binding Detection by Single-Wall Carbon Nanotube Network Junctions Fabricated with Surface Programmed Assembly Method

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Abstract

This paper presents the results of protein binding detection using single wall carbon nanotube network junctions as the transducer channel. The carbon nanotube junctions have been fabricated by using surface programmed assembly, where the carbon nanotubes align themselves and assemble in predefined patterns without using any external force or unconventional process like high temperature treatment, etc. This process leaves exposed the SiO_2 surface on which the carbon nanotubes are assembled. This enables biotin immobilization by simple chemistry. When streptavidin is injected, the strong binding between biotin and streptavidin is detected by the carbon nanotube junction, yielding a highly sensitive streptavidin sensor for protein binding studies.

Keywords: Surface programmed assembly, Single walled carbon nanotube, Biotin, Streptavidin, Protein binding, Nanobiosensor

Introduction

Carbon nanotubes have been widely investigated for diverse applications since their first discovery[1]. This wide interest in carbon nanotubes has been driven by their remarkable electrical, mechanical, chemical and structural properties. Especially, the significant conductance change that single-wall carbon nanotubes (SWNTs) show to gas-phase chemicals[2] has initiated a similar effort in the field of biomolecule detection in aqueous phase. Many reports show high sensitivity and selectivity results when SWNT-based nanosensors are used to detect biomolecules[3]. The high sensitivity derives from the molecular structure of SWNTs, where the nanotube consists only of surface atoms without any bulk atoms inside it, making the material extremely sensitive to exterior stimuli. The high selectivity derives not on the properties of the SWNTs themselves but on the properties of the biomolecules we are handling. For coupling the binding event to the conductance change of the SWNTs, some immobilization process of biomolecules is essential. Thus, in principle, we can obtain a high selectivity and sensitivity nanobiosensor by combining a sensitive nanoscale transducer and a biomolecule immobilization method adequate for the experiment at hand.

The protein binding detection using SWNTs junctions have been widely investigated due to their relatively direct interaction and accessibility to interpretation[4,5]. Also, several methods of immobilizing biomolecules to SWNTs for the purpose of protein detection have been proposed[6]. Current investigation shows that SWNT-based biosensors are remarkably sensible to the detection of proteins. However, despite of all these remarkable results, there has been also a major obstacle in real-life application of these nanosensors, mainly due to the lack of a reliable mass fabrication method of SWNT junctions[7].

Recently, a linker-free assembly method of SWNTs has been reported[8]. This technique utilizes the surface programmed assembly method, where the massive integration of SWNTs in any desired pattern is guided by surface molecular patterns[9]. These patterns guide the selective adsorption of the SWNTs onto bare SiO_2 surface. Here we can apply further functionalization using silane chemistry to immobilize proteins.

Herein we report the results of protein detection by utilizing the well-known streptavidin-biotin binding mechanism by utilizing the SWNT junctions fabricated by surface programmed assembly method as the transducer channels. The binding of biotin and streptavidin is one of the most extensively studied phenomena in life science. Chicken avidin and bacterial streptavidin are proteins widely utilized in a number of applications in life science, ranging from purifica-

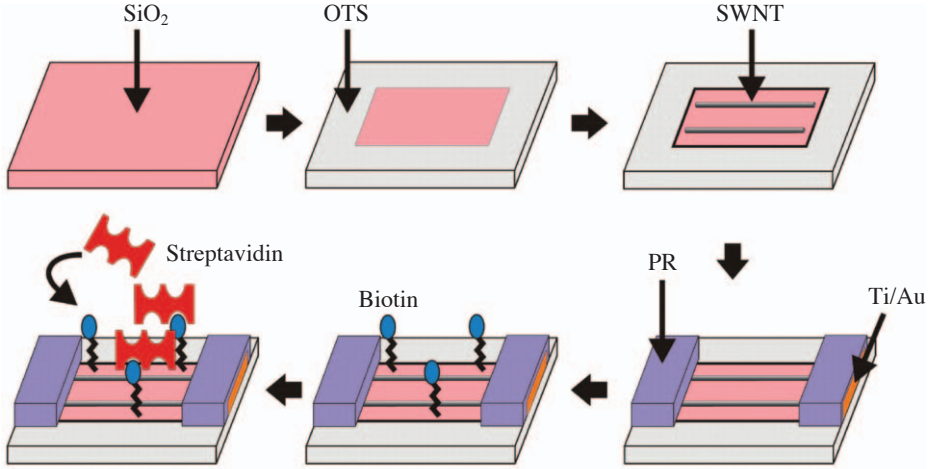

Figure 1. Schematic of fabrication and sensing scheme. (a) Bare SiO_2 surface (b) Molecular patterning with photolithography; (c) CNT assembly by surface programmed assembly method; (d) Electrode deposition and polymer passivation; (e) Biotin immobilization; (f) Streptaviding binding to immobilized biotin.

tion and labeling techniques to diagnostics, etc. Streptavidin-biotin technology relies on the extremely tight and specific affinity between streptavidin and biotin (dissociation constant, $K_d = 10^{-14} \sim 10^{-16}$ M).

For immobilizing biotin molecules around SWNTs, a generic method applicable to diverse kinds of nanotubes or nanowires was used. This method will open a wide range of application in the field of protein detection using SWNT junctions.

Results and Discussion

Biotin Immobilization

Figure 1 shows the fabrication method of the SWNT junction, the immobilization of biotin, and the sensing scheme of streptavidin. First, a predefined photoresist (PR) pattern is obtained on the SiO_2 substrate by conventional photolithography process. The sample is then dipped in octadecyltrichlorosilane solution (1 : 500 v/v in anhydrous hexane). Afterwards, the PR is removed by acetone and clean bare SiO_2 is exposed. The sample is dipped in SWNT suspension, where SWNTs are dispersed in *o*-dichlorobenzene, and the SWNTs are selectively adsorbed on the bare SiO_2 surface. Au/Pd electrode is deposited and additional PR layer is used to passivate the electrodes to reduce leakage current[10]. Afterwards, The sample is dipped in 3-aminopropyltriethoxysilane (APTES) solution (1 : 500 v/v in ethanol) to functionalize the bare SiO_2 surface with amine groups. The sample is then dipped in biotin solution. The ester group of the biotin molecule reacts with the amine group of the APTES and forms a covalent bond, resulting in immobilized biotin molecules. The sample is kept in pH 7.4 buffer during the experiment to retain bioactivity. Biotin solution was prepared by dissolving 5 mg of (+)-biotin N-hydroxysuccinimide ester (Sigma, USA) into 200 L of N, N-dimethylformamide and 2.5 mL of 2-propanol[6]. The solvent ratio was adjusted in order to prevent dissolution of the polymer layer passivating the electrodes. Afterwards, doses between 100 pM to 1 M of streptavidin were sequentially injected and the detection limit was observed. A control experiment was performed to verify the existence of nonspecific binding effect of streptavidin.

Conductance Change of Carbon Nanotube Junctions

Electrical properties were measured for each junction, and junctions with similar resistance around 1 MΩ were selected and used for detection. A drain-source voltage V_{ds} of 0.1 V was applied and conductance change was measured using a semiconductor parameter characterization system (Keithley 4200, USA). A 50 μL drop of 1 mM pH 7.4 phosphate buffer salne (PBS) solution was placed over the junction before a predefined amount of streptavidin (Sigma, USA) dissolved also in 1 mM pH 7.4 PBS was injected. Also a Pt pseudo-electrode was used to reduce the ambient noise and apply a constant $V_g = -0.5$ V to operate the device in a constant liquid gate condition[11].

Figure 2 shows the detection data of streptavidin. First, a control experiment to verify the existence of non-specific binding effect of streptavidin to SWNT junction was performed. The experiment showed the existence of non-specific binding between SWNTs and streptavidin molecules when the streptavidin concentration exceeds 1 μM. Figure 2(a) shows the slight decrease of conductance when 1 μM of streptavidin was injected to a SWNT junction with no biotin immobilized, implying an upper detection limit where the high concentration of streptavidin affects directly

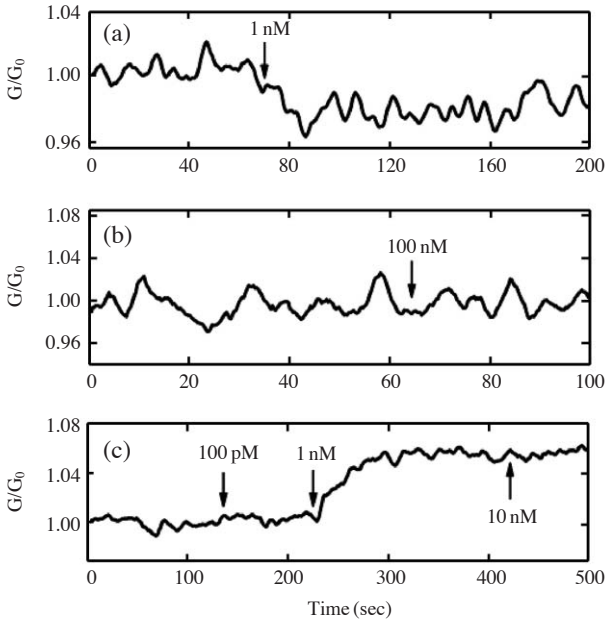

Figure 2. Binding effect of streptavidin (a) injection of 1 μM with no biotin immobilized; (b) Injection of 100 nM streptavidin in the absence of biotin; (c) Injection of 1 nM streptavidin in the presence of immobilized biotin

with the carbon nanotube junction. Similar trend of decreasing conductance when 1 μM of streptavidin is injected was obtained repeatedly for other similarly prepared five junctions. The SWNT junction has a p-type semiconductor property, showing a decrease of I_{ds} with positive V_g. and the effect of nonspecific binding of streptavidin to SWNTs can be considered in several aspects. First, the adsorbed proteins can act as scattering centers, which decrease the mobility of the holes and hence decrease the conductance. Second, the amine groups in the amino acid sequence could contribute electrons to the SWNTs, decreasing effectively the number of holes[12]. Figure 2(b) shows the conductance change when 100 nM of streptavidin was injected. This shows that the effect of nonspecific binding can be neglected when the concentration of the injected streptavidin is less than 100 nM. Considering these results, the experiment was performed by adding a given amount of 20 μL of increasing concentration of streptavidin in the range of 100 pM and 100 nM, increasing the concentration of the dose ten times between doses. Figure 2(c) shows the conductance change when three sequential doses of different concentration were injected. When 100 pM was injected, there was very little change in the conductance. When 1 nM of streptavidin was injected, about 6% of conductance change was detected. However, when 10 nM of streptavidin was injected additionally, little change was observed. From this experiment, we could find that once a sufficient amount of streptavidin is injected, almost every binding site becomes occupied such that further injection of additional streptavidin gives little change to the conductance. This somehow reflects the strong binding coefficient between biotin and streptavidin, and the saturation behavior study could be used in estimating the binding strength between two unknown biomolecules.

Non-contact AFM images were taken to investigate the role of the immobilized biotin in the overall binding event and hence in the sensing mechanism. The AFM junctions were taken in air after their respective process and extensive rinsing with D.I. water when needed. Figure 3(a) shows the area between the Au/Pd junctions, where the network of SWNTs is adsorbed on SiO_2 bare surface. Figure 3(b), which is the case when there is no biotin immobilized and 1 nM streptavidin was injected, shows a striking contrast to Figure 3(c) and (d), which correspond to the case with biotin immobilized with 1 nM and 1 mM streptavidin injection, respectively. This difference is clear evidence that immobilized biotin is essential in selective binding of streptavidin.

Conclusions

In this paper, we successfully fabricated a protein sensor, where the binding event between free streptavidin and immobilized biotin was monitored by observing the conductance change induced by the protein binding. We could identify the upper limit concentration of 1 μM of streptavidin, where nonspecific binding of streptavidin to the SWNTs begins to affect the conductance change. A detection limit around 1 nM was observed. However, this detection limit is expected to be enhanced by lowering the noise level and introducing signal amplification schemes. A 6% of conductance change was observed when 1 nM of streptavidin was injected. In immobilizing biotins, we didn't use any nanotube-specific chemistry. Since we used a very generic method of immobilizing proteins, this shows that SWNT based network junctions can be applied in the detection of diverse other kind of proteins. This will open the way toward a great usability in the study of proteins and protein sensors henceforth.

Methods

Materials

(+)-biotin N-hydroxysuccinimide ester, strepta-

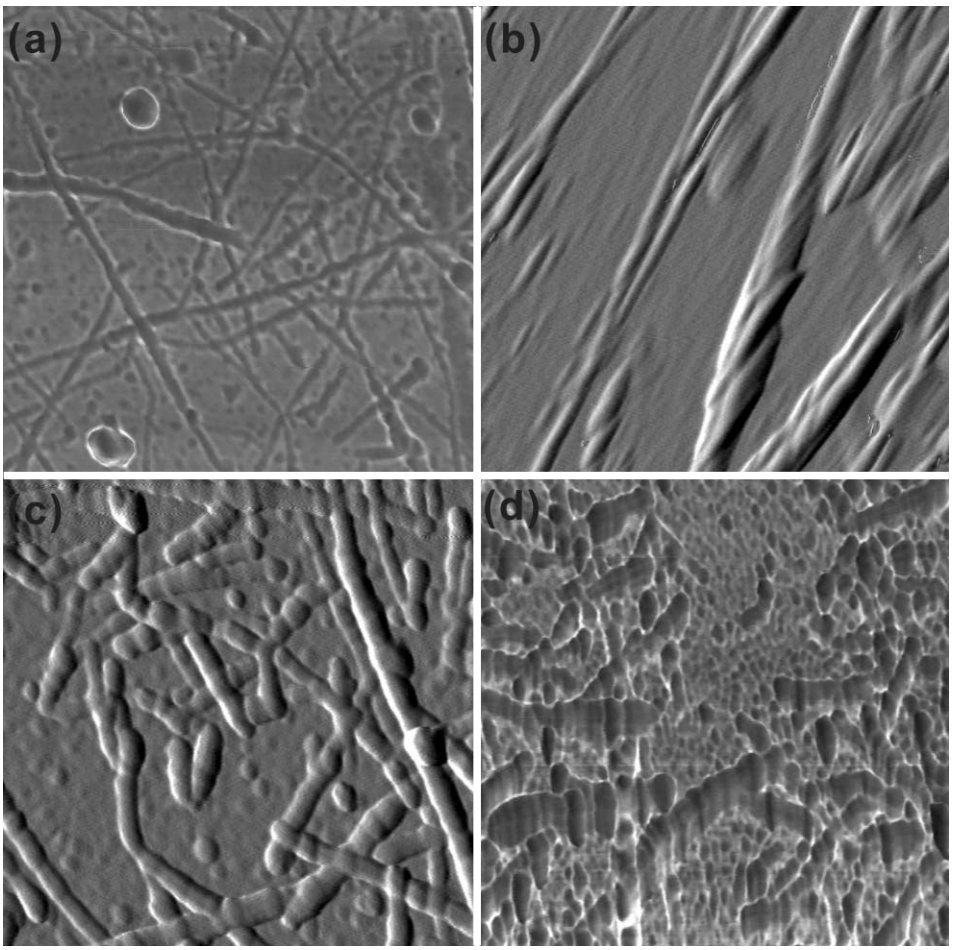

Figure 3. AFM images (a) CNTs adsorbed on SiO_2; (b) After injection of 1 nM streptavidin to a junction with no biotin immobilized; (c) After injection of 1 nM streptavidin to a junction with biotin immobilized; (d) After injection of 1 μM streptavidin to a junction with biotin immobilized. All : mages are 1 μm size.

vidin from *streptomyces avidinii*, octadecyltrichlorosilane, 3-aminopropyltriethoxysilane were obtained from Sigma-Aldrich. Purified SWNTs were purchased from Carbon Nanotechnologies Inc. (USA). All chemicals were used without further purification. The SiO_2 wafers were purchased from WaferMarket.com. The oxide thickness was 500 nm.

Carbon Nanotube Assembly

Purified SWNTs were dispersed in 1, 2-dichlorobenzene with ultrasonic vibration around 1 hr. The SWNT suspension concentration was 0.1 mg/mL. For SWNT assembly, the molecular patterned wafer was dipped in SWNT solution for 10 sec, and then rinsed thoroughly with 1, 2-dichlorobenzene and N_2 dried.

Electrode Deposition

After SWNT assembly, photoresist pattern is made by photolithography. Afterwards, 10 nm of Pd and 30 nm of Au were deposited by e-gun evaporator. The sample undergoes lift-off process, and another photolithography process yields PR passivation patterns on the electrodes.

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