

Final Report – AFOSR Nanoscience and Technology Initiative

Efficient fluorescence based protein chip using pseudo 3D single-walled carbon nanotube film

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Abstract

We have achieved two types of biomolecular sensors, colorimetric protein chips and label-free electrical sensors using high yield of single-walled carbon nanotubes (SWNTs). First, pseudo 3-dimensional SWNT films coated with carbonyldiimidazole-Tween20 (CDI-Tween20) surfactant demonstrated as a facile platform for fluorescence based protein chip. Highly selective protein bindings of biotin-BSA+SA (streptavidin) and SpA (protein A)+IgG (Immunoglobulin G) pairs, as well as those of small molecules such as FLAG peptide+anti-FLAG and biotin-SA. In this system, the geometry of pseudo 3-dimensional high yield of SWNTs preserves protein shapes intact, therefore increases the efficiencies of specific bindings. Furthermore, CDI-Tween20 mediates effective immobilization of probe proteins through covalent linkage, as well as prohibition of nonspecific bindings. By avoiding bovine serum albumin (BSA) which has been generally used as a biomolecular blocking agent during the protein chip manufacturing, it has been possible to reduce process steps, quenching of interaction signals from small molecules, and background noise.

We also have fabricated ultrahigh sensitive electrical protein sensors using single-walled carbon nanotube-field effect transistors (SWNT-FETs) which contain increased Schottky contact area. A simple fabrication technique utilizing thin shadow mask and thermal evaporation at tilted angles allowed metal to penetrate underneath of the mask efficiently. Hence, thin and wide metal to SWNT contact regions are obtained, which could accommodate more proteins comparing to the typically fabricated SWNT-FET devices by photolithography. Direct protein adsorption of SpA and specific binding of hCG+anti β -hCG pairs on SWNT-FET changed more than 1% of conductance change at 1 pM concentration without NSB. These new SWNT-FET devices have increased the detection limit about four orders of magnitude compared to the previous devices.

I. Efficient BSA-free fluorescence protein chip using pseudo 3D SWNT platform

Inquires of high throughput (HTP) analysis have accelerated rapid progresses in genomics and proteomics. Microarray technology combined with fluorescence detection method is one of the crucial and popular tools for HTP analysis of divergent biological events. Microarray techniques

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14. ABSTRACT

We have achieved two types of biomolecular sensors, colorimetric protein chips and label-free electrical sensors using high yield of single-walled carbon nanotubes (SWNTs). First, pseudo 3-dimensional SWNT films coated with carbonyldiimidazole-Tween20 (CDI-Tween20) surfactant demonstrated as a facile platform for fluorescence based protein chip. Highly selective protein bindings of biotin-BSA+SA (streptavidin) and SpA (protein A)+IgG (Immunoglobulin G) pairs, as well as those of small molecules such as FLAG peptide+anti-FLAG and biotin-SA. In this system, the geometry of pseudo 3-dimensional high yield of SWNTs preserves protein shapes intact, therefore increases the efficiencies of specific bindings. Furthermore, CDI-Tween20 mediates effective immobilization of probe proteins through covalent linkage, as well as prohibition of nonspecific bindings. By avoiding bovine serum albumin (BSA) which has been generally used as a biomolecular blocking agent during the protein chip manufacturing, it has been possible to reduce process steps, quenching of interaction signals from small molecules, and background noise. We also have fabricated ultrahigh sensitive electrical protein sensors using single-walled carbon nanotube-field effect transistors (SWNT-FETs) which contain increased Schottky contact area. A simple fabrication technique utilizing thin shadow mask and thermal evaporation at tilted angles allowed metal to penetrate underneath of the mask efficiently. Hence, thin and wide metal to SWNT contact regions are obtained, which could accommodate more proteins comparing to the typically fabricated SWNT-FET devices by photolithography. Direct protein adsorption of SpA and specific binding of hCG+anti hCG pairs on SWNT-FET changed more than 1% of conductance change at 1 pM concentration without NSB. These new SWNT-FET devices have increased the detection limit about four orders of magnitude compared to the previous devices.

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have been successfully applied to DNA sequencing, i.e. DNA chip, on which probe oligonucleotide strands are immobilized. Because of high stability of immobilized oligonucleotides and specific interactions with incoming target DNAs, DNA chip has been successfully utilized to reveal the ordered sequences in genes. On the contrary, protein chip has been suffered from unwanted nonspecific binding (NSB) owing to relatively weak affinity among probe protein and incoming proteins. For minimizing NSB of protein, bovine serum albumin (BSA) is most popularly used as a protein blocking material. BSA involved protein chips, however, frequently lead to ambiguous results not only due to its size and common biochemical nature as a protein, but also because of the complexity in pre- and post-treatment steps, such as protecting and quenching reactions. Therefore, it is highly demanded to develop a BSA-free protein chip substrate.

In this project, we demonstrate a BSA-free protein chip using 1,1'-carbonyldiimidazole activated Tween20 (CDI-Tween20) functionalized high yield single-walled carbon nanotube (SWNT) film which is directly grown on a SiO₂/Si substrate by chemical vapor deposition (CVD) method. Tween20 and its derivatized forms have been reported as a good "interfacing molecule" between carbon nanotubes and protein molecules in terms of facile immobilization and efficient NSB prohibition¹. After the passivation of CDI-Tween20 to the SWNT film, SWNT is ready to accommodate probe proteins while the bare SiO₂/Si surface still repels NSBs of proteins. Furthermore, it is also probable that the SWNT substrate may enhance the preservation of protein shape. Due to its high aspect ratio and pseudo 3D local network structure, SWNT film may provide the smallest contact area for the protein immobilization, which will minimize the shape deformation of protein molecules.

Experimental Section : 1) High yield SWNT growth on SiO₂/Si substrate : Iron(III)-hydroxylamine nanoclusters were spontaneously formed on SiO₂/Si substrate by immersing a substrate into 10 ml of deionized water in which 10 µl of 10 mM FeCl₃·6H₂O(aq) and 100 µl of 40 mM NH₂OH·HCl(aq) were sequentially added for total 3 min. This process was repeated twice to increase the population of nanoclusters. Iron(III)-hydroxylamine nanoclusters were then calcined at 800° in air to convert them into iron oxide nanoparticles. The growth of SWNT on the SiO₂/Si substrate containing iron oxide nanoparticles was performed by chemical vapor deposition (CVD) method at 950° for 5 min under the controlled gas flow rate of 1000/500/20 sccm of CH₄/H₂/C₂H₄, respectively. Successful growth of high yield pure SWNTs was confirmed by a simple resistance measurement (5 ~ 40 kΩ)².

2) Preparation of CDI-Tween20/SWNT substrate: 1,1'-carbonyldiimidazole-activated Tween20 (CDI-Tween20) was prepared by the reaction of Tween20 (2.25ml, Bio Basic Inc.) and 1,1'-carbonyldiimidazole (1.09g, Aldrich) in 6.08 ml of DMSO at 40° for 2 h³. In order to remove excess CDI and DMSO, the product solution was extracted with ethyl ether (Aldrich) resulting in yellow precipitation. Ethyl ether and residual DMSO was finally evaporated from the precipitates *in vacuo* overnight. The CDI-Tween20 was then functionalized on the sidewalls of SWNT through the van der Waals interaction by simply soaking a SWNT film substrate into the diluted CDI-Tween20 solution (1 wt% in water) for 1 h.

3) Preparation of biotinylated BSA : Biotinylated BSA (biotin-BSA) was prepared by the reaction of BSA (40 mg, Sigma) with EZ-Link[®] Sulfo-NHS-LC-Biotin (1 mg, Pierce) in 0.5 ml of PBS at 25° for 1 h and then dialyzed against 20 mM HEPES/NaOH at 4°.

4) Concentration control of proteins : Probe proteins including Protein A (SpA, derived from *Staphylococcus aureus*, Zymed[®]), biotinylated BSA(biotin-BSA), EZ-Link[®] Biotin-LC-PEO-

Amine (biotin, Pierce) and 3X FLAG[®] peptide (N-Met-Asp-Tyr-Lys-Asp-His-Asp-Gly-Asp-Tyr-Lys-Asp-His-Asp-Ile-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-C, Sigma) are diluted to 250 µg/ml in 10 mM PBS buffer (pH 7.4). Target proteins, Cy3-labeled rabbit anti-mouse Immunoglobulin G (Cy3-IgG, Zymed[®]), Cy5-labeled Streptavidin (Cy5-SA, Zymed[®]) and ANTI-FLAG[®] M2 Monoclonal Antibody-Cy3 Conjugate (Cy3-antiFLAG, Sigma) are diluted to 20 µg/ml in 10 mM PBS buffer.

5) Spotting and detection of proteins by using microarrayer and laser scanner : Probe proteins were spotted on CDI-Tween20/SWNT substrate in a 4×4 spot array using a protein microarray dispenser (Cartesian[™]). The sample was then incubated for 3 h and washed with PBS buffer (10 mM, pH 7.4) for 1 min then with distilled water. After drying in N₂, the substrate was reacted with 20 µg/ml of target protein for 1 h then washed with PBS buffer and distilled water for 9 min. Using a laser scanner (arrayWoRx^{®e}), protein spot arrays were characterized by monitoring their shapes and fluorescence intensities. During the experiments, humidity and temperature were controlled at 80 % and 20 ~ 25°, respectively (Figure 1).

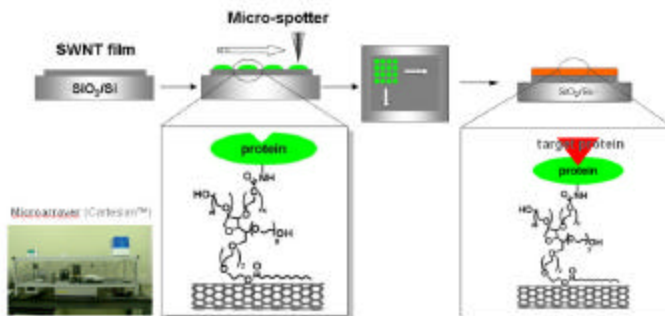


Figure 1. A schematic view of CDI-Tween20/SWNT platform for microarray applications.

Colorimetric protein chip using BSA-free CDI-Tween20/SWNT substrate: For the successful

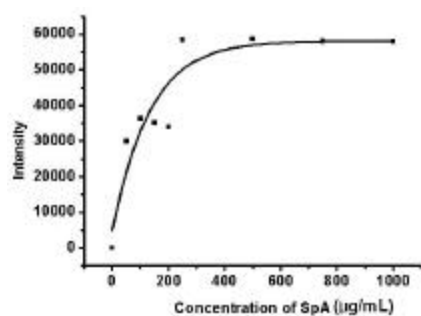


Figure 2. . Fluorescence intensity-concentration curve obtained from specific bindings of Cy3-IgG (20 µg/ml) and SpA immobilized at various concentrations. This graph indicates that SpA immobilization reaches to the saturation level at ca. 250 µg/ml.

application of CDI-Tween20/SWNT substrate for general protein chip application, it is necessary to confirm reproducible and homogeneous formation of protein micro-spots. The best spotting condition was decided from test spotting (4×4 array) of 20 µg/ml of Cy3-labeled IgG (Cy3-IgG) at various conditions such as concentration, incubation time on the CDI-Tween20/SWNT substrate (not shown). Next, we studied specific interactions of two sets of proteins, SpA + Cy3-IgG and biotin-BSA + Cy5-labeled SA (Cy5-SA) pairs. The optimal concentration for the immobilization of probe protein was selected from a fluorescence vs. concentration curve (Figure 2). Fluorescence intensities measured from 20 µg/ml of Cy3-IgG bound to SpA immobilized on CDI-

Tween20/SWNT substrate at various concentrations were systematically monitored and it was found that the saturation occurred at around 250 $\mu\text{g/ml}$.

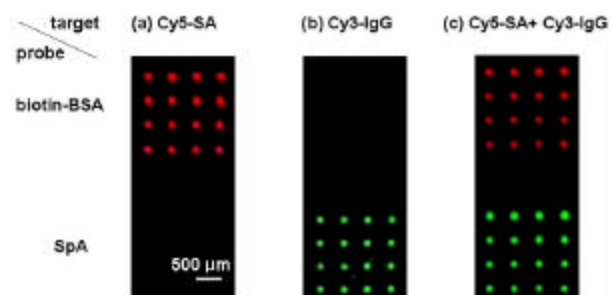


Figure 3. Fluorescence images after reaction of substrates containing biotin-BSA (top region) and SpA (bottom region) with (a) Cy5-SA, (b) Cy3-IgG, and (c) mixed solution of Cy5-SA and Cy3-IgG.

For the study of specific and nonspecific interactions, biotin-BSA (250 $\mu\text{g/ml}$) was first spotted in 4x4 spot array on the top region of the CDI-Tween20/SWNT substrate followed by SpA (250 $\mu\text{g/ml}$) spotting on the bottom region. After incubation, the sample reacted with 20 $\mu\text{g/ml}$ of Cy5-SA. As shown in Figure 3a, only biotin-BSA spots show fluorescence (top region) in red color due to their specific binding to Cy5-SA while SpA spots do not bind to Cy5-SA as the spots remain dark (bottom region). An

opposite observation was found when the substrate was reacted with 20 $\mu\text{g/ml}$ of Cy3-IgG solution. Only SpA spots show green fluorescence originated from their specific binding to Cy3-IgG (Figure 3b, bottom region) while the biotin-BSA spots remain dark (Figure 3b, top region). Specific/NSB efficiency was very high, i.e. ca. 60,000 fluorescence intensity counts for specific binding of SpA and Cy3-IgG but lower than ca. 100 for NSB measured from the area where biotin-BSA and Cy3-IgG reacted. The fluorescence intensity from individual spot was calculated by the software provided by ImaGene®.

Cross reactivity was also investigated by immersing a substrate containing the same arrays of probe proteins, i.e. biotin-BSA at the top region and SpA at the bottom region, into a solution of 1:1 mixture of Cy5-SA and Cy3-IgG at their final concentration of 20 $\mu\text{g/ml}$ each. As shown in Figure 3c, both red spots from Cy5-SA/biotin-BSA and green spots from Cy3-IgG/SpA specific bindings were well resolved. This indicates that no significant nonspecific cross reaction occurred. All these results suggest that the immobilized proteins on CDI-Tween20/SWNT substrate specifically bind to their counterparts.

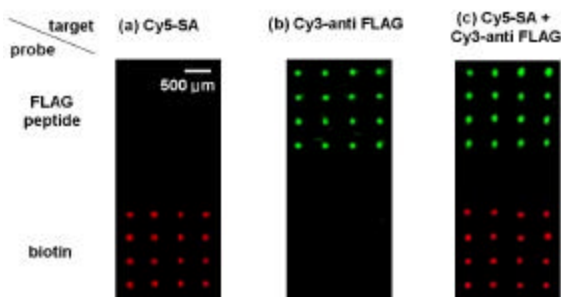


Figure 4. Fluorescence images after reactions of substrates containing FLAG (top region) and biotin (bottom region) with (a) Cy5-SA, (b) Cy3-antiFLAG, and (c) mixed solution of Cy5-SA and Cy3-antiFLAG.

Successful colorimetric small molecule sensor using CDI-Tween20/SWNT substrate: The BSA-free CDI-Tween20/SWNT substrate has been also attempted for the immobilization and specific recognition of small biomolecules. For this demonstration, we prepared another two pairs, FLAG + Cy3-labeled anti FLAG (Cy3-anti FLAG) and EZ-Link® Biotin-LC-PEO-Amine (biotin) + Cy5-SA. Similar to the previous experiments, 250 $\mu\text{g/ml}$ of FLAG and biotin were spotted on the top and the bottom region, respectively. After reaction

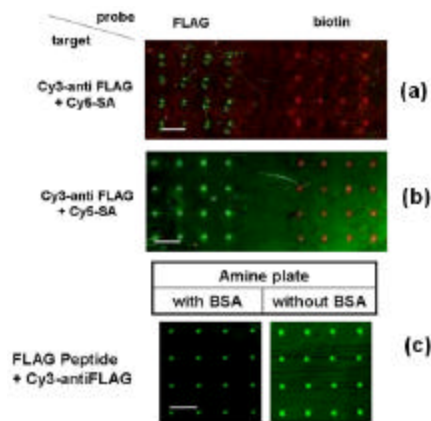


Figure 5. Fluorescence images representing SWNT population effects. FLAG and biotin are immobilized then reacted in a mixed solution of Cy3-antiFLAG and Cy5-SA on a (a) CDI-Tween20/low yield SWNT substrate and (b) high yield SWNT substrate *without* CDI-Tween20. (c) Specific and nonspecific bindings of FLAG peptide (probe) with Cy3-antiFLAG (target) on the commercially available SuperAmine (ArrayIt™) glasses. The scale bars indicate 500 μ m.

SuperAmine slides then reacted with Cy3-antiFLAG in the absence of BSA, the spot/background fluorescence intensity ratio is substantially increased, more than twice to when BSA is applied (Figure 5c). While BSA indeed provides efficient NSB prohibition, it also diminishes fluorescent spot sizes (>75% in area), which entails one of the previously discussed drawbacks of using BSA, shielding of small biomolecules (Figure 5c).

The proposed mechanism for the surface passivation with CDI-Tween20 and its effect on the NSB prohibition is as follows; the CDI-Tween20 on SWNT readily reacts with incoming proteins via covalent bonding while the unreacted CDI-Tween20 on SWNT is simultaneously quenched by water during the incubation for 3 h under 80% of humidity. Hence, the NSB of protein is sufficiently prohibited on SWNT

with 20 μ g/ml of Cy5-SA (Figure 4a), Cy3-antiFLAG (Figure 4b) and a mixture of those two (Figure 4c), it was certainly observed that the target proteins recognized their probe biomolecules selectively.

The CDI-Tween20/SWNT substrate provides high specificity in protein recognition with negligible extent of NSB throughout the substrate. It reveals that SWNT and CDI-Tween20 play an important role not only for efficient covalent coupling of proteins but also for excellent NSB prevention. Indeed, high yield SWNT (5 ~ 40 k Ω) is critical for the reliable protein spot formation because no adequate spots were found from a bare SiO₂/Si substrate (not shown) or from a low yield SWNT substrate (200 ~ 500 k Ω) functionalized with CDI-Tween20 (Figure 5a). A control experiment of microarray assay at the absence of CDI-Tween20 on high yield SWNT film also showed increased background signal and cross reactivity due to the increased NSB (Figure 5b). Our BSA-free SWNT based protein chip has been also directly compared with a state-of-the-art protein chip substrate, SuperAmine (ArrayIt™). When small peptide FLAG molecules are immobilized on

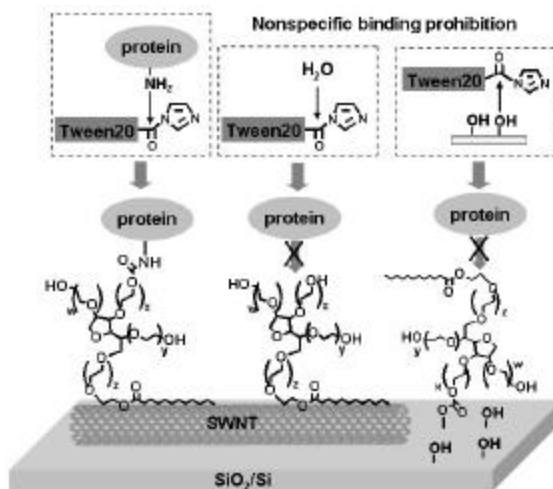


Figure 6. Functionalization of SWNT on SiO₂/Si substrates with CDI-Tween20. 1) covalent coupling of probe proteins to SWNTs (left), 2) nonspecific binding (NSB) prohibition on SWNT (middle), and 3) NSB prohibition on a bare SiO₂/Si containing hydroxyl groups (right).

(Figure 6). Even though the portion is quite small, there is still bare SiO_2/Si region where SWNT does not cover. As shown in Figure 6, the bare SiO_2/Si surface is also protected via covalent coupling of imidazolyl carbamate group of CDI-Tween20 with hydroxyl groups on SiO_2/Si surface during the CDI-Tween20 coating period, which finally forms NSB repelling Tween20 layer. Note that the size of hydrophobic chain of the CDI-Tween20 molecule is much smaller comparing to that of hydrophilic branches

Summary : CVD-grown high yield SWNT film has been successfully demonstrated as an effective substrate for microarray protein chip applications without BSA involvement. High specific/nonspecific protein binding discrimination ratio was observed from CDI-Tween20 passivated pseudo 3D SWNT film substrates. Not only for the conventional probe-target protein interactions, has the CDI-Tween20/SWNT protein chip been also applied for small probe biomolecules, such as FLAG peptides. Due to its simplicity in the preparation with an advantage as a BSA-free system, it is highly expected that the CDI-Tween20/SWNT protein chip will provide opportunities for the extensive and detail studies about divergent biomolecular interactions.

II. Ultrasensitive electronic protein sensor using SWNT-FET devices including increased Schottky contact areas

Since the first demonstration of an electrical nanobiosensor using silicon nanowire-field effect transistor (SiNW-FET) devices⁴, there have been many attempts to exploit label-free electrical sensing systems using similar types of nanomaterials. Single-walled carbon nanotubes (SWNTs) are one of the promising candidates due to their high biocompatibilities and well-established knowledge about their device properties.

However, one uprising issue of such a system is sensitivity. In the case of SWNT-FET device, the reliable detection limit for the sensing of proteins or protein-protein interactions is ca. 100 pM to 100 nM¹⁵. The low sensitivity of SWNT device is intimately related to the sensing mechanism and the corresponding device geometries such as Schottky barrier (SB) modulation effect⁵ as well as chemical gating effect⁶. The SB effect will dominate especially when the isoelectric point (pI) of a protein is close to the pH of the reaction media⁵.

In this project, we have substantially improved the sensitivity of SWNT-FET devices for protein-protein interactions by modifying the geometry of the devices, increased Schottky contact area. This has been achieved by evaporating metals for source and drain electrodes using a shadow mask at a tilted angle during the device fabrication.

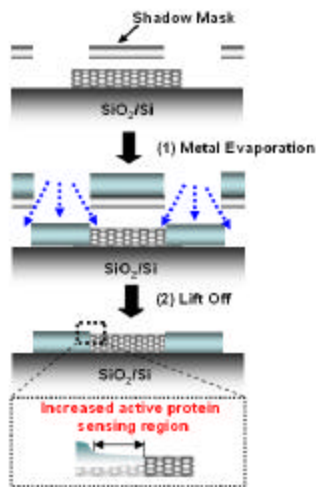


Figure 7. A schematic view representing fabrication of SWNT-FET devices using a shadow mask.

Experimental Section : For the fabrication of improved sensitivity of SWNT-FET devices, network SWNTs (avr. diameter = 1.7 nm) were synthesized on SiO_2/Si wafers by

chemical vapor deposition (CVD) method which is similar above-mentioned process (refer to experimental section of 'I. Efficient BSA-free fluorescence protein chip using pseudo 3D SWNT platform'). Thin aluminum shadow masks having 200 ~ 300 μm width were suspended on SWNT area in which a 15 μm thick Al foil was manually cut into a strip (200 μm $\times$ 1 cm), then placed onto a SWNT networks containing SiO_2/Si substrate of which edges were blocked by double-sided tapes. The strip was gently placed on the double-sided tape, which provides ca. 50 μm gap between the substrate and the mask. After that, the sample was transferred into a thermal evaporator chamber equipped with a 23° tilted sample stage (Figure 7). During the evaporation of Cr (15 nm) followed by Au (30 nm), metals were guided to penetrate underneath the shadow mask. Such devices show very little gate field dependence, i.e. pseudo-metallic transport characteristics (Figure 8a). This abnormal transport property seems due to suppressed field efficiency shielded by percolated metals covering the surfaces of SWNT channels, which is also indicative for the formation of thin and wide Schottky contact area (Figure 8b).

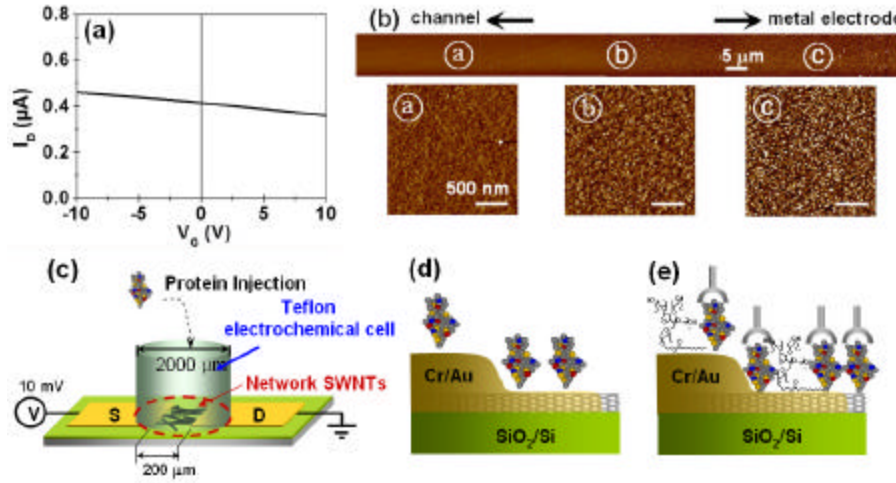


Figure 8. (a) Network SWNT-FET devices of which I - V_g curves represent pseudo metallic characteristics ($V_{ds} = 10$ mV). (b) AFM images of metal electrodes fabricated by angle-evaporation using a shadow mask. In contrast to the microfabricated device, angle-evaporation with a shadow mask generates metal electrodes with a gradient thickness. High magnification images of (a), (b) and (c) clearly depict significant differences in metal film thickness depending on the position of the device. For a visual clarity, electrode devices without SWNTs were examined. (c) Schematic view of a homemade Teflon electrochemical cell used for protein sensing. (d) Nonspecific protein adsorption on the devices and (e) specific target protein bindings to probe proteins immobilized on a Tween20-protected device.

All the experiments were performed in a homemade Teflon electrochemical cell which is designed to expose both Schottky contact area and SWNT channels to protein solutions (Figure 8c). The devices were installed into the electrochemical cell, then filled with phosphate-buffered saline (PBS, 10 mM, pH = 7.4) solution while 10 mV of bias voltage (V_{ds}) was continuously applied between source and drain electrodes. When the devices start to show a steady current, proteins at specific concentrations were injected into the cell using a micropipette (Figure 8d). In order to compare the sensitivity of our devices with the ones fabricated by photolithography⁵, we examined same proteins used in the previous reports, such as Protein A (SpA, derived from *Staphylococcus*

aureus, Zymed®), mouse antibody β -hCG (anti β -hCG , Lab Vision), human chorionic gonadotropin (hCG, Sigma), and rabbit Immunoglobulin G (IgG, Sigma).

In specific binding proteins sensing case, probe proteins were immobilized on the devices by immersing as-fabricated devices into the concentrated probe protein solutions for 3 h followed by the treatment with Tween20 (0.05 wt% in PBS solution) for 2 h. The Tween20 treatment protects unoccupied sites of the device from NSBs. Once the devices are stabilized, target proteins of various concentrations were injected stepwise (Figure 8e).

Efficient nonspecific bindings on modified SWNT-FET devices : The devices with increased Schottky contact area have shown high sensitivity with 1 pM detection limit for nonspecific

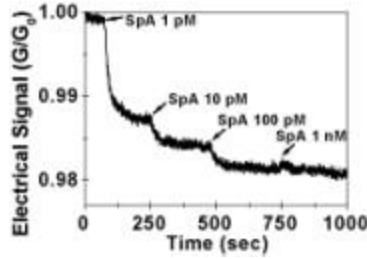


Figure 9. Electrical sensing of nonspecific binding of SpA using network SWNT-FET device ($V_{ds} = 10$ mV).

bindings of proteins as well as specific bindings of protein pairs. This is 10^4 time increased detection limit comparing to the reported similar nanotube based devices, especially for specific protein-protein interactions. First, we tested the sensitivity of devices for nonspecific bindings of various proteins.

All the examined devices have shown significant conductance drops ($> 1\%$ conductance change) upon nonspecific adsorption of proteins at as low as 1 pM concentration. Figures 9 show representative conductance drops upon the additions of SpA at various concentrations. Although slightly different extents of conductance drop have been observed from devices to devices, twelve out of

fifteen devices have shown 1pM sensitivities with greater than 1 % conductance drop. The rest three devices have also shown conductance drops at 1 pM of protein solutions but with high noise level and less than 1 % conductance change.

Efficient specific bindings using modified SWNT-FET : The current devices have also shown 1 pM sensitivity level for specific bindings of protein pairs. Figures 10 shows systematic conductance drop upon the representative specific recognitions of hCG by anti β -hCG. The devices show apparent conductance drops at 1 pM of target proteins. From the fact that control injections of PBS and bovine serum albumin (BSA) have not changed the conductance, it is

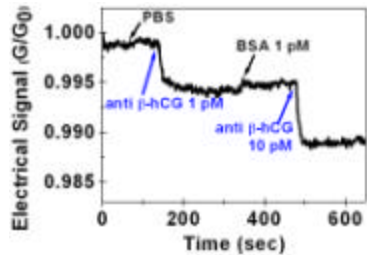


Figure 10. Electrical sensing of specific protein pair of hCG (probe) – anti- β -hCG (target) using network SWNT-FET device ($V_{ds} = 10$ mV).

confirmed that the conductance drops are solely attributed to the specific bindings between probe and target proteins.

The substantially increased sensitivity is primarily due to the thin and wide Schottky contact area on which relatively more numbers of proteins can adsorb at low concentration, resulting in prompt modulation of metal work function of the devices. The formation of thin and wide metal coating by shadow mask angle-evaporation has been confirmed by AFM, which clearly shows the evaporated metals having a gradient thickness over a wide range of area. (Figure 8b) Control experiments tested with devices containing identical CVD grown

SWNTs but fabricated without angle-evaporation generally show normal $I-V_g$ curve of network style of SWNTs (>50% drop, Figure 11a) and respond to protein adsorptions at >10 nM (Figure 11b). Moreover, it is also critical for protein to be adsorbed on thin metal Schottky contact area since there has been no response when a micro-droplet of PBS and proteins are placed on thick metal surfaces which are 45 nm vertically away from the Schottky contact interface, even at very high protein concentration (> μ M). (Figure 11c)

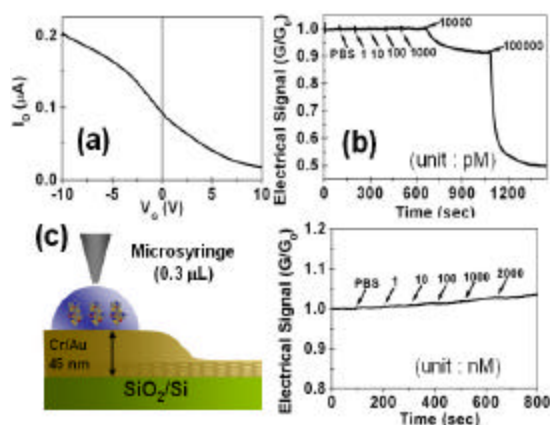


Figure 11. (a) $I-V_g$ curve of SWNT-FET devices fabricated using a shadow mask without angle-evaporation. (b) Electrical sensing of nonspecific binding of SpA ($V_{ds} = 10$ mV) from the device shown in (a). The numbers in the graph (b) indicate the concentration of injected SpA in μ M. (c) Schematic view of microsyringe-assisted protein adsorptions on a metal electrode surface which are 45 nm vertically away from SWNTs (left) and conductance changes of the device upon the nonspecific adsorption of PBS and SpA (right). The numbers on the graph indicate the concentration of injected SpA in nM.

Along with the increased Schottky contact area, internanotube Schottky contacts may further increase the sensitivity. Since the network SWNTs grown in high yield by CVD naturally populate both semiconducting and metallic nanotubes, the Schottky point contacts are formed in high population where semiconducting and metallic SWNTs are crossed⁷.

Summary : We have fabricated highly sensitive network SWNT-FET devices which have successfully detected both nonspecific adsorptions of proteins and specific protein-protein interactions at 1 pM concentrations. The increased sensitivity is mainly accredited to the increased thin and wide Schottky contact area, which has been achieved by evaporating electrode metals using a shadow mask on a tilted angle sample stage. The accomplishment of highly sensitive SWNT devices is expected to accelerate the progress towards the realization of nanoscale and label-free electronic biosensor systems.

Publications

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