DNA-mediated self-assembly of carbon nanotubes on gold

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Abstract. This report presents the use of disulfide-modified single-stranded DNA (ssDNA) to form DNA self-assembled monolayers (SAMs) and mixed DNA-carbon nanotube (CNT) hybrids SAMs on gold substrates. Mixed DNA-CNT SAMs are composed of DNA, mercaptohexanol (MCH) and DNA-CNT aggregates. Both, DNA-CNT and DNA areas of the mixed SAMs were analyzed and compared to traditional DNA SAMs. The results suggest the formation of a more compact and densely packed monolayer of DNA-CNT in comparison with DNA. The use of DNA-CNT hybrids to form SAMs on gold substrates might represent a new approach to improve the immobilization of DNA strands on gold, and might therefore help with the development of enhanced DNA sensors.

1. Introduction

Self-assembled monolayers (SAMs) of disulfide- or thiol-modified single stranded DNA (ssDNA) are commonly used in the fabrication of DNA sensors, since they confer stable and cost-effective immobilizations. Nevertheless, the immobilization of DNA strands on solid substrates, such as gold, is not a simple process. One of the major concerns in the fabrication of DNA sensors using the self-assembly technique is reproducibility, given that the stability, density and organization of the SAMs depend on several factors, such as the forces of attraction between the immobilized molecules-including the interaction between the terminal groups-, the forces between the surface and the binding group, and the structure of the solid substrate. In addition, previous work has demonstrated that non-specific adsorption of DNA strands on solid substrates can occur and might affect the efficiency and reproducibility of the DNA sensor, since the hybridization efficiency is higher for strands perpendicular to the surface than for the ones lying flat on the substrate [1]. Instead of the traditional approach to solve this problem- which uses mercaptohexanol (MCH) to compete with the non-specifically adsorbed DNA for the empty spaces on the surface- we propose the immobilization of DNA-wrapped CNTs on gold substrates to minimize the non-specific adsorption of DNA strands.

DNA-wrapped CNTs solutions prepared with an excess of disulfide-modified-DNA (containing a mercaptohexyl linker), will be used to prepare mixed monolayers on gold, which will be composed of DNA-CNT complexes, DNA strands and MCH molecules (obtained when the S-S bond in the disulfide-modified DNA is broken upon chemisorption on the gold surface). These mixed SAMs will be analyzed and compared to traditional DNA SAMs, and it will be determined whether they offer some advantages over DNA SAMs on gold. The DNA-CNT mixed monolayers on gold substrates might serve as the basis for the design and fabrication of novel DNA sensors.

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2. Experimental Methods

2.1. Materials
All chemicals were purchased from Sigma-Aldrich, were used as received and are of analytical grade. Aqueous solutions were made with nanopure water with an 18MΩ-cm resistivity and degassed with nitrogen prior to any electrochemical measurement. Aqueous solutions of DNA-CNT hybrids were prepared by a procedure similar to the one used by Zheng and coworkers [2]. As-received SWNTs (from Carbon Nanotechnologies) were mixed with disulfide-modified ssDNA, 5’ HO-(CH2)6-S-S-(CH2)6-AGAAGGCCTAGA 3’ (from Synthegen, LLC), in a 0.05 M Na2HPO4/0.1 M NaCl buffer (pH 7.00) and sonicated for 1 hour in an ice-bath. The mixture was diluted to prepare dispersions with different DNA concentrations, which were centrifuged for 30 minutes to remove insoluble impurities. Results for the characterization of the DNA-CNT hybrids are published elsewhere [3]. The DNA-CNT complexes had lengths that ranged from 40 to 100 nm, with an average length of (70 ± 20) nm, and an average diameter of (2.5 ± 0.2) nm.

Polycrystalline gold electrodes from Bioanalytical Systems (BAS) Inc., with a 1.6 mm diameter, and quartz microbalance polycrystalline gold sensor crystals, with a 2.54 cm diameter, were employed as substrates for the experiments. The electrochemical polishing pre-treatments and the procedure used for roughness determination of the working electrodes are described elsewhere [3, 4]. The reference and auxiliary electrodes were Ag | AgCl | 3M KCl and a platinum mesh, respectively.

Self-assembled monolayers were prepared by immersing a clean gold electrode in a vial containing the desired species to be immobilized (i.e. DNA, DNA-CNT). Nitrogen was used for degassing the solutions, and the vial was sealed with paraffin and left unperturbed during the modification period. After the 24-hours immobilization period, the electrodes were removed from the solution, cleaned with nanopure water, and dried with a nitrogen stream.

2.2. Instrumentation
A Nanoscope IIIa-Multimode atomic force microscope from Digital Instruments, with a scanning probe microscope controller equipped with a He-Ne laser (638.2 nm) and a type E scanner, was used for the atomic force microscopy (AFM) analysis. All the samples were analyzed in tapping mode, using a phosphorous (n) doped Si cantilever from Veeco.

The electrochemical measurements were carried out in a conventional three-electrode cell, and N2-purging for 15 minutes was done prior to any analysis. The measurements were performed on a Princeton Applied Research (PAR) 273A potentiostat/galvanostat, controlled with a PAR M270 Research Electrochemistry Software. All potentials are reported in reference to the Ag | AgCl | 3M KCl electrode.

X-ray photoelectron spectroscopy (XPS) spectra were recorded in a PHI 5600ci spectrometer, using an Al Kα monochromatic source (350 W, 15 kV), and at a takeoff angle of 45°. Survey and multiplex spectra were obtained with pass energies of 187.85 eV and 58.70 eV, respectively. All the binding energies reported were corrected using the signal for the carbon contamination peak (C 1s) at 284.5 eV as an internal standard.

3. Results and Discussion
Previous reports by our group have shown that CNTs can be vertically attached to gold substrates when they are wrapped with disulfide-modified ssDNA [3, 5, 6]. Mixed monolayers formed by ssDNA, MCH (used as the protecting group in the disulfide-modified ssDNA) and DNA-CNT hybrids have been obtained and characterized. A typical AFM image of the mixed SAM obtained, showing aggregates of DNA-CNT complexes with heights of up to 100 nm, is presented in figure 1. In addition, aggregates of DNA strands in a fully extended conformation can be found in the mixed SAM, at heights of 15 nm, corresponding to a 6 nm-height for the DNA strand, after subtracting the height of the gold substrate.
DNA aggregates in a CNT-free area of the mixed DNA-CNT SAM, were further studied by AFM and compared to traditional DNA SAMs. Figures 2a and 2b show a DNA SAM and the section analysis for the DNA aggregate. Figures 2c and 2d show the image and the section analysis for a DNA SAM in a DNA-CNT-free area from the 0.01 µM DNA-CNT SAM sample. A comparison of the height profiles show that the DNA SAM area from the DNA-CNT sample contains a higher surface coverage of DNA in a fully extended configuration. The images also show that the DNA-CNT form more ordered DNA aggregates than the traditional DNA SAM.

X-ray photoelectron spectroscopy was used to study the nature of the interaction between the disulfide-modified-ssDNA-CNT and the gold substrate in DNA-CNT and DNA SAMs. High-resolution XPS spectra for the Au 4f region (figure 3) show a decrease in the signal intensity for the 0.01 µM DNA-CNT-modified sample (c), compared to bare gold (a), as expected for a SAM-modified surface, since the molecules on the SAM make difficult the detection of the Au 4f photoelectron. This signal attenuation is not as large as one would expect for a CNT covered surface, due to the fact that only ~1% of the surface was covered with DNA-CNTs. In addition, the signal intensity for the DNA-CNT SAM (c) is lower than the intensity for the DNA SAM of equal DNA concentration (b), which is
expected for surfaces modified with longer molecules, such as CNTs, but might also suggest a higher surface coverage of DNA strands.

High-resolution XPS binding energy spectra for the S 2p region of DNA SAMs (a) and DNA-CNT SAMs (b) are shown in figure 4. The lower intensity for DNA-CNT SAM S 2p signal might be caused by an attenuation of the signal due to the presence of CNTs, which are larger molecules than DNA and might, therefore, make more difficult the detection of the S 2p electron. Spectral deconvolution of the S 2p emission band for each modified surface (figure 4, inset) exhibited two doublets with binding energies (BEs) of 161.8 eV and 163.8 eV for the S(2p\textsubscript{3/2}) peaks, which also suggests modification of the Au surface with sulfur-containing functionalities. The peak separation for each doublet was 1.1 eV, and the ratio between the S(2p\textsubscript{1/2}) and S(2p\textsubscript{3/2}) peak areas was approximately 1:2, as previously reported [7]. The S(2p\textsubscript{3/2}) binding energy (BE) value of 161.8 eV is in good agreement with the BE values attributed to the S-Au bond for alkanethiols [7] and thiol-modified DNA SAMs [8,9]. The S(2p\textsubscript{3/2}) peak at a BE of 163.8 eV was assigned to protected DNA molecules (RS-S-ssDNA) that are non-specifically adsorbed to the surface, and this BE correlates well with previously reported values for protected DNA molecules immobilized on gold [10].

![Figure 3: High-resolution XPS spectra for the Au 4f signal of (a) bare gold, (b) 0.01 µM DNA SAM on Au, (c) 0.01 µM DNA-CNT SAM on Au.](image)

![Figure 4: High-resolution XPS spectra for the S 2p signal of (a) 0.01 µM DNA SAM on Au, and (b) 0.01 µM DNA-CNT SAM on Au. Inset: Spectral deconvolution of the S 2p emission band for the DNA-CNT SAM.](image)

A comparison study between DNA-CNT and DNA SAMs was performed using a DNA concentration of 0.01 µM for the immobilizations, and cyclic voltammetry (CV) in K\textsubscript{3}Fe(CN)\textsubscript{6} (1mM in 0.1 M KCl; scan rate: 50 mV/s) was used to characterize the modified electrodes. The comparison between bare and modified electrodes is shown in figure 5, and the ΔE values for the samples were 80 mV, 270 mV and 350 mV for bare gold, a DNA SAM and a DNA-CNT mixed SAM, respectively. The voltammograms for the modified electrodes exhibited larger peak separation, a decrease in the peak current, and changes towards a more sigmoidal shape, which correspond to the typical electrochemical behavior of SAM-modified electrodes [11, 12]. The DNA-CNT-modified sample shows a larger decrease in current than the DNA-modified electrode, suggesting that the DNA-CNT SAM has fewer pinholes and, therefore, might be more compact than the DNA SAM. Additionally, the peak separation is larger for the DNA-CNT mixed SAM, which indicates that the kinetics of electron-transfer are slower due to a higher barrier produced by the DNA-CNT SAM.
4. Conclusions
The use of DNA-CNT hybrids to form mixed SAMs on gold has shown some advantages over traditional DNA SAMs. CV, XPS and AFM results suggest the formation of a more compact and densely packed monolayer of DNA-CNT in comparison with DNA. One possible explanation for the higher immobilization efficiency of DNA-CNT hybrids SAMs compared to DNA SAMs is that when CNTs are present in solution, there might be a decrease in the non-specific adsorptions of the DNA on gold, due to the strong interactions between the DNA bases and the CNTs. In a wrapping configuration, the DNA bases interact with the graphitic structure of the CNTs via π-π adsorptions [2], which might limit the interactions between the DNA bases and the gold surface.

Considering the higher efficiency of DNA-CNT hybrids immobilization on gold, compared to the immobilization of DNA strands on pure DNA SAMs, the methodology previously presented, which is used to immobilized these hybrids by the self-assembly technique, in combination with electrochemical desorption and detection techniques, can serve as the basis for the fabrication of novel sensors and devices.

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