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Selective Functionalization of In_2O_3 Nanowire Mat Devices for Biosensing Applications

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Nanomaterials, such as carbon nanotubes (CNTs)[1] and nanowires (NWs),[2] have been employed in nanoscale biosensing devices.[3] The detection of DNA and proteins is an important goal, where the diagnosis and treatment of genetic diseases and the identification of infectious agents are of central importance.[4] Nanoscale biosensing devices give very low detection limits for biological molecules and biologically important ions, largely due to their high surface-to-volume ratio, such that even low levels of analyte binding markedly affects the properties of the nanomaterial, for example, its conductivity. Selectivity to a given analyte has been accomplished by anchoring an analyte-specific recognition group to the surface of the CNT or NW.

Indium oxide (In_2O_3) NWs have been used to fabricate both chemical sensors and biosensors.[5] They can work as valid alternatives to CNTs or Si NWs. Unlike Si NWs, In_2O_3 NWs do not have a native oxide coating between the active semiconductor and the bound analyte. Bare In_2O_3 NWs, when previously employed as gas sensors, showed ultrahigh sensitivity down to parts per billion level, fast response, and fast recovery time.[5a] To detect biomolecules the In_2O_3 NW, surfaces must be coated with analyte binding groups. To this end, we have extended the surface chemistry reported by Mrksich and co-workers[6] toward selectively functionalizing In_2O_3 NWs with DNA strands, as confirmed using voltammetric and fluorescence studies. This procedure can be readily generalized to selectively immobilize biological molecules onto various metal oxide (e.g., SnO_2 and ZnO) NW surfaces. A related process for Si NW functionalization has been reported,[7] in which the linking molecules are anchored to silicon NW surfaces using UV radical photoactivation, followed by deprotection of the hydroquinone residue using BBr_3. Our approach achieves the In_2O_3 NW surface functionalization via a simple and mild self-assembling process, utilizing 4-(1,4-dihydroxybenzene)butyl phosphonic acid (HQ-PA). This eliminates the use of UV photoactivation and BBr_3, a very corrosive Lewis acid we have found to attack many metal oxide NWs.

We have generated a self-assembled monolayer (SAM) of HQ-PA on the In_2O_3 NW surface (Figure 1a). HQ-PA is an electrochemically active molecule containing a hydroquinone group that undergoes reversible oxidation/reduction at low potentials.[6b] Oxidized HQ-PA (Q-PA) reacts with a range of functional groups, which can be easily incorporated into biomolecules and other materials, such as thiols, azides, cyclopentadienes, and primary amines. Here, we have attached a thiol-terminated DNA, electrochemically addressing only the desired NWs (Figure 1b).

Derivatives of phosphonic acid are well-known to bind to indium tin oxide (ITO),[8] which has a surface composition very similar to that of In_2O_3 NWs. Therefore, preliminary studies were carried out

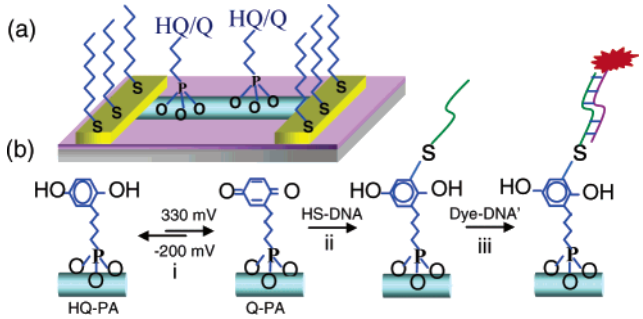

Figure 1. (a) Schematic representation of an In_2O_3 NW mat device (only one NW is shown for clarity). The NW is functionalized with a SAM of HQ-PA and is placed between two gold electrodes protected by a SAM of dodecane-1-thiol. (b) (i) The monolayer of HQ-PA, deposited on the In_2O_3 NW or ITO, can be reversibly oxidized to Q-PA in an electrochemical cell. (ii) Addition of the probe, thiol-terminated DNA (HS-DNA) to Q-PA. (iii) Attachment of complementary DNA strand (dye-DNA′) to the probe DNA.

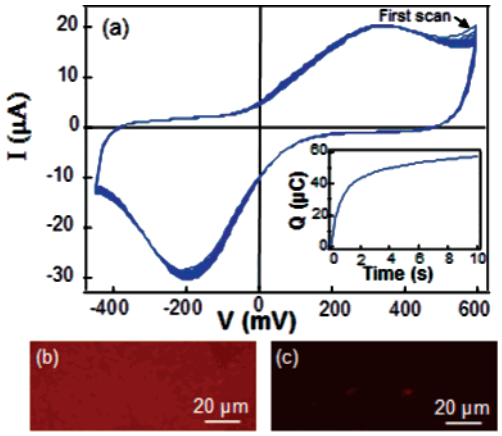

Figure 2. (a) Fifteen consecutive CVs showing the reversible oxidation/reduction of a SAM of HQ-PA on an ITO glass sheet. (Inset) Chronoamperometry shows the amount of charge necessary to oxidize a predefined area of HQ-PA: an average of 56.8 μC. (b) A fluorescence image of ITO surface that was oxidized to Q-PA, reacted with SH-DNA, and the DNA paired to its complementary DNA strand labeled with a fluorescence dye. (c) A fluorescence image of ITO with the HQ-PA monolayer went through the same DNA attachment procedure as that of the ITO sheet in (b), showing little or no DNA binding.

on ITO-coated glass. A monolayer of HQ-PA was deposited on freshly cleaned ITO surfaces by submerging the substrate into a 0.1 mM aqueous solution of HQ-PA for 16 h. The surface coverage and stability were characterized by electrochemical analysis. A cyclic voltammetry trace (CV) of a derivatized film is shown in Figure 2a. The film was scanned multiple times (shown for 15 consecutive scans) in the range from −450 to +600 mV. The oxidation wave is centered at +330 mV, and the reduction wave is centered at −200 mV (Figure 2a), which are in agreement with

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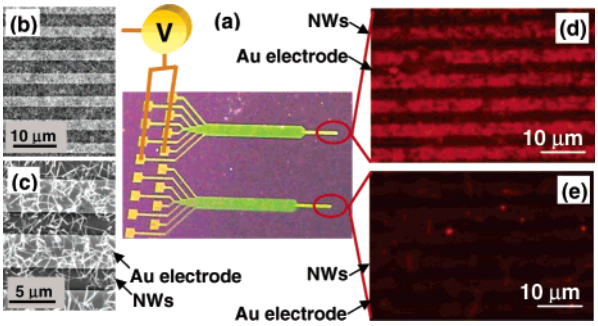

Figure 3. (a) A photograph of a NW mat sample contacted by two groups of electrodes. Only the HQ-PA attached to the NWs between the upper electrodes was converted to Q-PA. (b) An SEM image of the In_2O_3 NWs before functionalization. The brighter stripes are gold electrodes covering the NW mat. (c) The same sample imaged at higher magnification, where the NW mat is clearly visible. (d) A fluorescence image of the NWs with Q-PA taken after DNA attachment. The gold electrodes, passivated with an alkanethiol, appear dark under the fluorescence microscope. (e) A fluorescence image of the NWs with HQ-PA after DNA incubation. The NWs appear dark, indicating no DNA attached to HQ-PA.

previously published data.[6,7] Also, chronoamperometric measurements on a predefined area of the ITO sheet (0.57 cm^2) reveal that a charge of 57 μC is consumed for the complete oxidation of the HQ-PA SAM, at +400 mV (Figure 2a inset). This gives a surface coverage of 4.9×10^{-10} mol/cm^2 or 33.7 $Å^2$/molecule, indicating that HQ-PA forms a densely packed monolayer.[8,9] The potential of the cell was then brought to +450 mV and held for 5 s to create a monolayer of Q-PA (active state). A separate ITO sheet was held at −350 mV to ensure the complete reduction of the monolayer of HQ-PA (inactive state). Both ITO sheets were then submerged in a solution of a 10 μM thiol-terminated DNA[10a] for 2 h (solvent = PBS buffer containing a catalytic amount of triethanolamine, pH 7.40; Figure 1b, step ii). The ITO sheets were next incubated for 30 min in an aqueous solution containing the complementary DNA[10b] tagged with a red fluorescence dye (Figure 1, step iii). The ITO surface was rinsed with a PBS buffer (pH 7.00) containing 1 M NaCl to wash away any excess of complementary DNA. Fluorescence microscopy images of the activated (Figure 2b, bright) and inactivated (Figure 2c, dark) ITO sheets demonstrate the success of this selective functionalization strategy. This demonstrates that DNA binds only to the activated ITO sheet in a uniform manner. In control experiments using a mismatched DNA-dye,[10c] the ITO sheet appears dark, indicating no pairing (not shown).

Having established the successful functionalization strategy on the ITO surfaces, the same reaction sequence was applied to In_2O_3 NWs. A mat sample of In_2O_3 NWs (average diameter of 10 nm and length of 3 μm) was grown on a SiO_2/Si wafer, followed by photolithography and metal (Ti/Au, 3 nm/50 nm) deposition to pattern an electrode array. The resultant device is shown in Figure 3a. We chose to work with NW mat devices as they have numerous advantages, such as great reliability, high sensitivity, and ease of fabrication when compared to individual NW devices. Figure 3b,c shows typical SEM images of the NW mat sample used in this study. Multiple nanowires were found bridging the Au electrodes. A SAM of HQ-PA was created on a freshly cleaned In_2O_3 NW mat sample using the same procedure as that for the ITO sheets. The In_2O_3 NW device was placed into the electrochemical cell and completely reduced to HQ-PA. To prevent the thiol-terminated DNA from attaching to the gold electrodes, the sample was treated with dodecane-1-thiol after HQ-PA SAM formation. This resulted in the formation of a SAM of a C_{12} alkyl chain on the Au electrodes surface (Figure 1a). The device was then placed into an electrochemical cell with both electrodes submerged in the electrolyte but with potential applied across only select Au electrodes (upper electrodes in Figure 3a). The potential of the cell was held at +450 mV for 5 s. In this way, the monolayer of HQ-PA coating on specific NWs was oxidized to Q-PA. Then, the entire device was submerged in the SH-DNA solution for 2 h, resulting in the selective, covalent linkage of DNA to the NWs with Q-PA.

Fluorescence studies were used to confirm the selective functionalization of the In_2O_3 NW array. Typical fluorescence images from similar nanowire devices with Q-PA and HQ-PA, which have been treated with the complementary DNA strand containing a fluorescent dye label, are shown in Figure 3d and e, respectively. In Figure 3d, the gold electrodes appear as dark lines, whereas the NW mat with Q-PA, derivatized with DNA, appears as a bright network. In contrast, the NWs with HQ-PA, which went through the same DNA treatment, do not show any fluorescence, as seen in Figure 3e. This demonstrates that there is no DNA binding to the NWs with HQ-PA.

In summary, we have demonstrated selective functionalization of an array of In_2O_3 NW-based devices by electrochemically activating their surfaces and then immobilizing single-strand DNA. This can be considered a key step for the future fabrication of large-scale biosensor arrays or chips for inexpensive multiplexed detection. The methodology described here may also be adapted to selectively functionalize individual NW devices.

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Supporting Information Available: Experimental details for the synthesis of HQ-PA, device fabrication, surface functionalization, and fluorescence microscopy. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Oligonucleotides base sequence. (a) Thiol-terminated DNA: (5′-HS-GCT TTG AGG TGC GTG TTT GT-3′); (b) complementary DNA with dye: (5′-ALEX546-ACA AAC ACG CAC CTC AAA GC-3′); (c) mismatched DNA with dye: (5′- ALEX546-ACA AAC ACT TTC CTC AAA GC-3′).

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