

Supplementary Information to:

Electrically Addressable Biomolecular Functionalization of Carbon Nanotube and Carbon Nanofiber Electrodes

Chang-Soo Lee^{†§}, Sarah E. Baker^{†§}, Matthew S. Marcus[‡], Wensha Yang[†], Mark A. Eriksson[‡], and Robert J. Hamers^{*†}

[†]Department of Chemistry, University of Wisconsin, 1101 University Avenue, Madison, WI 53706, USA

[‡]Department of Physics, University of Wisconsin, 1150 University Avenue, Madison, WI 53706, USA

[§]These authors contributed equally to this work and should both be considered as first authors.

**Author to whom correspondence should be addressed. Email: rjhamers@wisc.edu*

Patterning and growth of carbon nanotube-modified electrodes:

Silicon(100) wafers with resistivity of .01 to .02 ohm-cm and a 0.5 μM thermal oxide grown on both sides were used as substrates for nanotube work. The nanotube contact pads were patterned in Shipley 1813 photoresist and developed using Shipley MF 321. Contacts were provided by evaporation of 50 nm of molybdenum followed by lift-off in acetone. The nanotube catalyst was patterned onto the molybdenum electrodes using electron beam lithography using a polymethyl methacrylate (2%, 950 K molecular weight, in anisole) resist (Microchem) that was spin coated onto the substrate at 4,000 RPM. This positive radiation resist was then exposed at select regions using a LEO Supra 55 VP SEM equipped with a Nabity electron beam lithography system and a dose of $220 \mu\text{C}/\text{cm}^2$ at 25 kV. These exposed sites over the microelectrodes were then selectively removed using a developer consisting of a 1:3 solution of methyl isobutyl ketone and isopropyl alcohol (Microchem), leaving a set of patterned Mo electrodes.

Single-wall carbon nanotubes were grown on the Mo electrodes via catalyzed thermal chemical vapor deposition. The catalyst, an alumina-supported Mo/Fe nanoparticle catalyst (First Nano) was spin-coated onto the substrate at 3,000 RPM; liftoff of the excess catalyst and resist was done in N-methyl pyrrolidone at a temperature of 60°C . Finally, the patterned chip was placed into a commercially available nanotube growth furnace (First Nano). The chemical vapor deposition was carried out at 900°C for 10 minutes using 400 sccm methane and 20 sccm hydrogen.

Patterning and growth of vertically-aligned carbon nanofiber bundles on electrodes:

The fabrication of the Vertically-Aligned Carbon Nanofibers (VACNFs) used Si(100) wafers with a 300 nm film of low-pressure chemical vapor deposited (LPCVD) silicon nitride as an insulator.

Molybdenum contacts were patterned as above. Electron-beam lithography was used with PMMA photoresist to define small small regions for deposition of catalyst; the catalyst consisted 20 nm titanium followed by 20 nm of Ni. Nanofibers were grown on the catalyst-patterned substrate using 4 torr of a mixture of acetylene (16 sccm flow rate) and ammonia (80 sccm) via DC Plasma-Enhanced Chemical Vapor Deposition (PECVD). With the sample as the cathode, 330 watts of power was applied using an Advanced Energy MDX 1K power supply for 12 minutes; the plasma heated the sample to approximately 700 °C. Electrically addressable functionalization of these VACNFs is using the same sequence of steps as the functionalization of the SWNTs electrodes described above.

Nanotube functionalization with aromatic nitro groups via diazonium chemistry:

After initial growth of carbon nanotubes (CNTs) and/or vertically-aligned carbon nanofibers (VACNs) on the Mo electrodes, the subsequent chemical modification steps were identical. In order to functionalize the electrically-addressable CNTs and VACNs on the chip with nitro groups, the chip was immersed in a 36 mM solution of 4-nitrobenzenediazonium tetrafluoroborate (Aldrich) in 1% sodium dodecyl sulfate (Promega) and shaken using a vortex mixer at room temperature for 24 hours. The chip was then rinsed by immersing and gently shaking in three fresh solutions each of deionized water and acetone. At this stage, the chip consists of molybdenum electrodes coated with SWNTs or VACNs, bearing reactive nitro groups.

Electrically-addressable electrochemical modification of specific nanotube-coated Mo electrodes:

In order to electrochemically “activate” a specific set of CNTs or VACNs on the chip by converting the nitro groups to amino groups, the associated molybdenum contact was connected to a conventional 3-electrode potentiostat using the sample as the working electrode. The other CNT/VACN-coated electrodes and a larger Pt foil were used as the counter-electrode, and a AgCl-coated Ag wire as the reference electrode. The reduction of the nitro-benzene groups on the carbon

nanotubes was simultaneously monitored and carried out using cyclic voltammetry on a Solartron 1287 electrochemical interface. The potential (reported vs. the Ag/AgCl reference electrode) was swept from a starting potential of -1.0 V down to -1.8 V, up to -0.2 V, and back to -1.0 V, at a rate of 200 mV/sec in a solution of 0.1 M KCl in a 90:10 solution of ethanol in deionized water. The reduction peak was typically observed between -1.0 and -1.5 V. Typically, each scan was repeated four times in order to verify the irreversible conversion of nitro-groups to amino group on the CNTs/VACNs. This step produces reactive amine groups only on the specific subset of CNTs/VACNs that are on the Mo electrode that was used as the working electrode; those on the other Mo electrodes remain nitro-terminated.

Modification of amine-modified nanotube electrodes with DNA oligonucleotides:

The amino terminated CNTs/VACNs were then made reactive toward 5' thiol terminated oligonucleotides by immersing the chip in a 1.5 mM solution of the heterobifunctional cross-linker sulfo-succinimidyl 4-(N-maleimidomethyl)cyclohexan-1-carboxylate (SSMCC, Pierce) in 0.1 M triethanolamine buffer (Aldrich), pH 7.0 for 20 minutes, exposing reactive maleimide groups. The chip was then rinsed with DI water. DNA oligonucleotides used here were 32 bases in length consisting of a repeated spacer of 15 thymine bases (T_{15}) followed by 16 bases for hybridization and were modified with the 5' thiol modifier C6 (Glen Research). After deprotecting the thiol modifier, the oligonucleotide was purified by chromatography and used immediately. To link the oligo to the maleimide-modified CNTs/VACNs, the oligo of interest (50 μ M in triethanolamine buffer, pH 7) was applied to the surface and kept in a humid chamber in the dark for two hours. The chip was then rinsed in 2xSSPE/0.2%SDS buffer (Promega, consisting of 2mM EDTA, 7 mM SDS, 300 mM NaCl, 20 mM NaH_2PO_4 with the pH 7.4) to remove excess DNA. At the end of this step, the nanotubes on a specific Mo electrode have been covalently bonded to a specific biomolecule, such as DNA oligonucleotide S1. To produce an array of *distinct* biologically-modified electrodes, the electrochemical reduction

(previous step) followed by the covalent linking (the current step) can be repeated with a different biomolecule of interest. In our work, we performed the procedure a total of 4 times, producing a 4-element array of electrodes with distinct oligonucleotides. However, it should be possible to repeat the process a very large number of times to produce a very high-density array of biomolecular recognition sites.

Confirmation of specific biomolecular recognition properties of DNA-modified nanotube electrodes:

To prepare the array of four different DNA sequences, the first region was electrochemically activated (i.e., the nitro groups were reduced to amine groups) and modified with the DNA oligonucleotide S1 as described above. The next region on the chip was then electrochemically activated and then functionalized with S2, and similarly for subsequent regions, producing a 4-element array of DNA-modified nanotube electrodes in which each nanotube electrode was modified with a different sequence of DNA. To check the selectivity of local chemical modification, the entire chip that was modified with the four different sequences of DNA at four different locations was then immersed in a mixture of all of the complementary DNA sequences, 5'-FAM-TT GCA GCT CGA TCG TT-3' (F1, complementary to S1) 5'-Cy3-TT GCT CCT GCA TCG TT-3' (F2, complementary to S2) 5'-Cy3.5-CG AAA GCT CGA TAA GC-3' (F3, complementary to S3) 5'-Cy5.5- CG ATT GCT CCT TAA GC-3' (F4, complementary to S4), 5 μ M of each in 2XSSPE/0.2%SDS buffer, for 20 minutes. As noted in the sequences of F1 through F4, each complementary sequence was modified with a different fluorescent tag. These were, specifically, 6-FAM amidite, Cy3 Phosphoramidite, Cy3.5 Phosphoramidite, and Cy5 Phosphoramidite. The fluorescent tags were purchased from Glen Research and the complete oligonucleotides were synthesized by the University of Wisconsin biotechnology center. The tags used have peak absorbance and emission wavelengths sufficiently distinct that each could be distinguished from the other on a single chip by fluorescence measurements. A fluorescence imager (Genomic Solutions GeneTac UC4x4) was used to generate a

fluorescence image of the differentially modified regions on the chip by successive scanning with lasers and optical filters matching the appropriate absorption and emission profiles of the individual dyes. The specific excitation and emission wavelengths are as follows: 488 nm excitation and 512 nm emission bandpass filter for the FAM dye, 532 nm excitation and 595 nm bandpass filter for Cy3, 594 nm excitation and 615 nm bandpass filter for Cy3.5, and finally 635 nm excitation and 695 nm bandpass filter for Cy5. The resulting images can then be represented as grayscale intensity maps (one for each particular set of absorption/emission wavelengths optimized for a specific dye), or can be represented using colors approximately representing the true colors of the dyes.