

DNA-functionalized single-walled carbon nanotubes

Chris Dwyer¹, Martin Guthold², Michael Falvo², Sean Washburn²,
Richard Superfine² and Dorothy Erie³

¹ Department of Computer Science, University of North Carolina at Chapel Hill,
NC 27599 USA

² Department of Physics and Astronomy, University of North Carolina at Chapel Hill,
NC 27599 USA

³ Department of Chemistry, University of North Carolina at Chapel Hill, NC 27599 USA

Received 31 July 2002, in final form 20 August 2002

Published 6 September 2002

Online at stacks.iop.org/Nano/13/601

Abstract

We present here the use of amino-terminated DNA strands in functionalizing the open ends and defect sites of oxidatively prepared single-walled carbon nanotubes, an important first step in realizing a DNA-guided self-assembly process for carbon nanotubes.

1. Introduction

The unique electrical properties of single-walled carbon nanotubes (SWNTs) make them good candidates for use in a self-assembling process that can controllably form electronic circuitry. Control over the assembly process may be derived from the selective binding of complementary DNA strands as in [1]. This work represents a step toward the DNA-guided assembly of carbon nanotubes by demonstrating that the well-known chemical pathway already discovered for attaching amino-terminal compounds to carbon nanotubes is also compatible with DNA functionalization [2]. Previous work in the field of nanotube–DNA self-assembly has focused on either non-covalent associations between the nanotubes and DNA molecule [3] or the self-organizational properties of a carbon nanotube/DNA system [4]. Other studies have explored the use of DNA self-assembly and frayed wire systems [5]. While these studies illuminate our understanding of self-organizing systems, our work focuses on developing a controllable assembly system. We hope that one day a high level of control will be possible by using the hybridization of covalently bound DNA strands on carbon nanotubes.

The single-walled carbon nanotube material, as formed by a laser ablation method, is first purified in nitric acid and then oxidized in a sulphuric and nitric acid mixture as described by Liu *et al* [2]. The product of this purification is a solution of open-ended nanotubes with terminal carboxylic acid groups. The carboxylic acid groups can be reacted with primary amine compounds by any of several condensation reactions [6]. The reactions couple the amine compound to the nanotube by way of an amide bond [7]. Figure 1 illustrates the basic chemical pathway that we have used in this work.

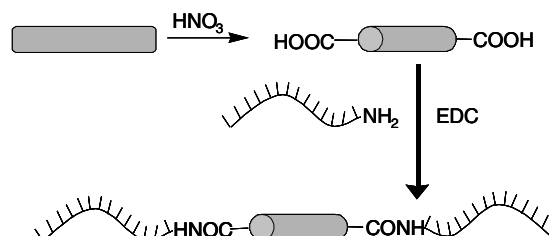


Figure 1. The DNA/nanotube reaction scheme. Capped nanotubes are oxidatively opened and then reacted with amine-terminated single-stranded DNA.

2. Materials and methods

Linking DNA strands to the nanotube requires specially prepared DNA strands. Amino-terminated DNA strands can be purchased from commercial suppliers⁴. For the first set of experiments described here, we used 10 μM amino-terminated DNA^{5,6} which had been purified using polyacrylamide gel electrophoresis (PAGE) by the vendor. Our second experiment used 51.1 $\mu\text{g ml}^{-1}$ of lambda-DNA extracted from bacteria⁷.

We have imaged the functionalization of the as-prepared single-walled carbon nanotube material (diluted in DMF) and the amino-terminated DNA strand oligo 1 and carboxylic-terminated DNA strand oligo 2 by ³²P radioisotope PAGE. Oligo 2 serves as our control for determining that the DNA

⁴ Operon Technologies, Inc., Alameda, CA; <http://www.operon.com>.

⁵ Oligo 1 sequence: 5'-NH₂-ATG GTG GAT AGG CGA CTC AAG GGC-3'.

⁶ Oligo 2 sequence: 5'-TTT TTT TTT TTT TTT TT-COOH-3'.

⁷ Lambda-DNA c1857 Sam7 isolated from *E. coli* strain W3350, 48502 bp in length, Promega Corp., USA.

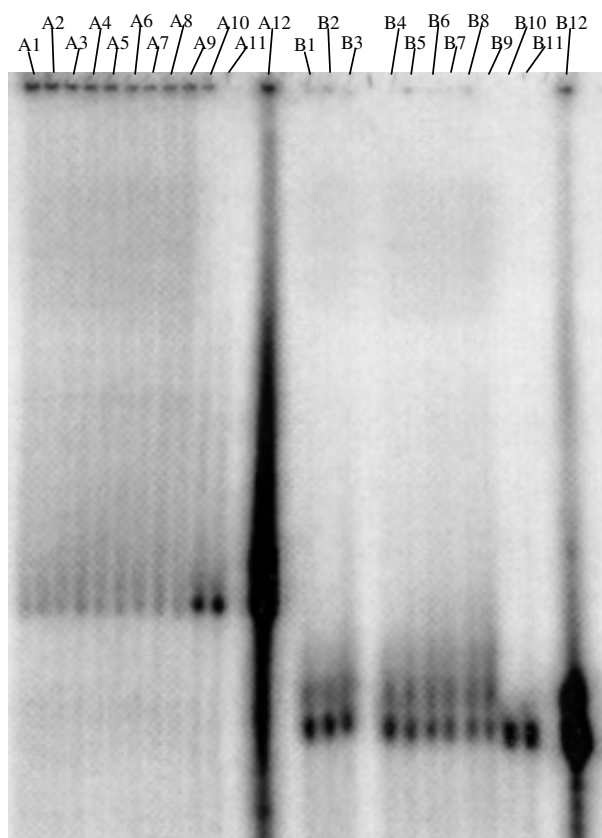


Figure 2. Polyacrylamide gel of reactions A1–A12 and B1–B12. The material bound at the top of A1–A9 indicates successful SWNT–DNA linking.

does not non-specifically interact with the nanotube material. We expect the low mobility of the SWNTs in the polyacrylamide gel to prevent any bound DNA from migrating at its normal rate through the gel. DNA strands migrate through a gel because their charged phospho-diester backbone interacts with the applied electric field. Shielding effects limit the force that can be applied to any strand and this means that strands will move at a rate that is inversely proportional to their length. The longer a strand is, the greater the number of interactions that strand will have with the gel, thus slowing its progress through the pores of the gel.

The DNA strands were first labelled with ^{32}P - γ -ATP using a T4 kinase enzyme⁸. Each of the following were added to a 0.5 ml microcentrifuge tube: 1 μl of oligo 1 or oligo 2 (10 μM), 10 μl ^{32}P - γ -ATP (3.3 μM , 3000 Ci/ μl), 1 μl T4-polynucleotide kinase (10 units/ μl), 6 μl 5 \times T4 reaction buffer (as described in the enzyme specification sheet, with the exception that no DTT (dithiothreitol) or Tris-HCl was used), and 12 μl nanopure H₂O (18.2 M Ω). The replacement of the Tris-HCl with PBS (phosphate-buffered saline) from the T4 kinase reaction buffer was required to prevent side reactions between the amine groups on Tris with the carbon nanotubes. Since Tris is smaller and more mobile in solution than the DNA strands, it will dominate the competition for carboxylic acid sites on the nanotubes. DTT was omitted from the reaction protocol to eliminate unnecessary additives. The labelling reaction was incubated at 37 °C for 2 h and then heat killed

⁸ T4-polynucleotide kinase and ^{32}P - γ -ATP, Amersham Pharmacia Biotech.

for 3 min at >65 °C. Following the heat kill, each DNA strand was purified from the kinase reaction using a phenol extraction.

The ^{32}P -labelled oligo 1 and diluted SWNT material were added to 50 mM EDC⁹ and incubated at room temperature for 24 h. Eight reactions were prepared using 1 \times , 2 \times , 4 \times , 8 \times , 16 \times , 32 \times , 64 \times , and 128 \times dilutions of 50 $\mu\text{g ml}^{-1}$ of SWNT in DMF and labelled as reaction A. Two control reactions were performed with one having no EDC and 50 $\mu\text{g ml}^{-1}$ SWNT (A9) and the other having no SWNT material (A10). The second set of reactions, reaction B, was identical to reaction A but used oligo 2 instead of oligo 1. Oligo 2 should have no primary amine groups for reaction with the SWNT material due to its sequence (poly-thymine has no primary amine groups).

The products of reactions A and B were loaded onto a 10% denaturing polyacrylamide gel using a loading buffer (30% glycerol, 25 mM EDTA, and 0.01% bromophenol blue and xylene cyanol). In addition to the previously described reactions, samples of the purified oligo 1 and oligo 2 (A11 and B11) were loaded as well as the kinase reaction product (A12 and B12) from which they were each purified. Each reaction was heated to 100 °C for 3 min before loading on the gel to denature any non-specific DNA binding. The gel was pre-run for 1 h using TBE, pH 8.9, buffer (Tris-HCl, borate, EDTA). Following the pre-run, the gel was loaded with samples and run for 3 h at \sim 900 V, 80 mA, with a closed-loop controller maintaining a constant power of 65 W by varying the plate voltage. The gel slab was then imaged by exposure to a radioisotopic imaging screen for 15 h. A screen scanner was then used to acquire a digital image of the gel.

We performed the second experiment to verify the reactivity of DNA with the SWNT material. We reacted lambda-DNA in place of the short oligos under the same conditions as described above. Lambda-DNA will react with the carboxylic acid groups on the SWNTs because of the primary amines found on the many A, G, and C nucleotides that the DNA contains. Approximately 10 mg EDC, 100 μl lambda-DNA, 300 μl SWNT material, and 600 μl nanopure H₂O were mixed and left to react at room temperature for 1 h. The relative insolubility of the SWNT material in H₂O compared to the modest solubility of DNA in pure H₂O reduces the chance of non-specific adsorption of the DNA to the SWNT material. 10 μl of the reaction product was deposited on UV-cleaned (6 min exposure, rinsed with nanopure H₂O, repeated twice) silicon with a native oxide layer. The silicon sample was then rinsed with nanopure H₂O and dried under a stream of dry N₂. The sample was then immediately placed into the load lock chamber of a Hitachi S-4700 SEM and pumped down to approximately 10⁻⁵ Torr. The sample was then imaged using typical SEM parameters for non-conducting samples. Figures 4 and 5 show that the lambda-DNA will readily form clusters with varied points of attachment to the SWNTs indicating that there may be multiple carboxylic acid binding sites at the end and along the sidewalls of the SWNTs.

3. Results

As expected from the dilution series of A1–A9, we see in figure 2 that there is a steady decline in the amount of ^{32}P -labelled DNA retained at the top of the lanes. Since we

⁹ 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; Sigma-Aldrich.

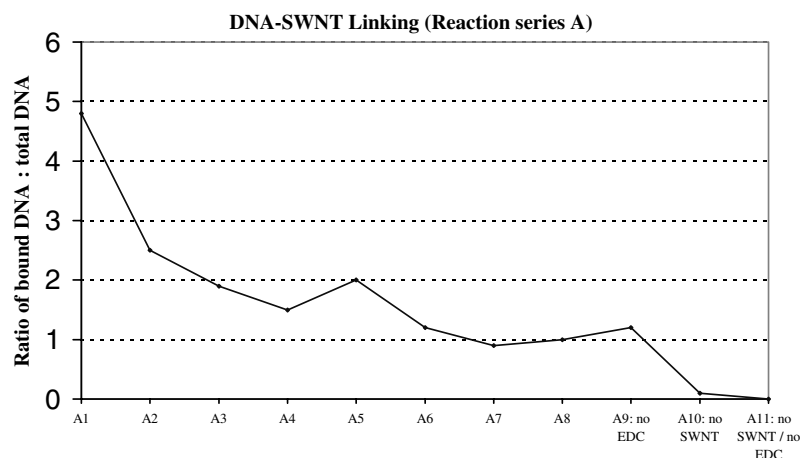


Figure 3. A plot of the ratio of DNA immobilized at the top of a well to the total DNA found in the well from reaction A. Reaction B appears to have little bound DNA.

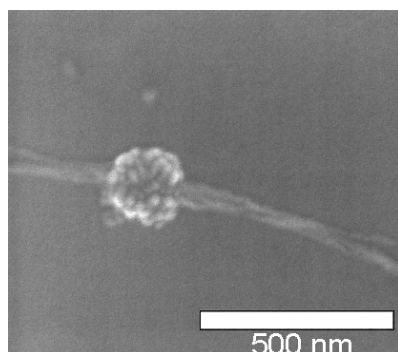


Figure 4. A lambda-DNA cluster attached to defect sites and ends of a SWNT bundle.

expect the reaction to greatly, if not completely, reduce the DNA mobility in the polyacrylamide gel, we expect to see a correlation between the mobility and SWNT concentration. Accordingly, as we reduce the concentration of SWNT material we see greater amounts of labelled DNA run down the gel lanes, indicating that less DNA is being immobilized. Lane A10 demonstrates the non-specific immobilization ‘background’ of the series since it has no SWNT material. Lanes A11 and A12 visualize the cleaned and original kinase labelling products. In particular, lane A12 indicates the importance of removing the by-products of the kinase reaction by the large degree of variation in strand mobility. This variation is probably due to any number of binding events between the kinase and DNA strands that reduce the strand mobility through the porous gel.

Because the oligo 2 used in reaction B has no primary amine groups, we do not expect it to react with the SWNT material. Further, since oligo 2 is shorter than oligo 1 (17 nucleotides versus 24 nucleotides) we expect oligo 2 have higher mobility in the gel. Lanes B1–B9 demonstrate this by the large amount of DNA moving through the gel with very little being immobilized at the top of the lanes. Lane B10 was used to characterize the background immobilization, near zero in this reaction, and B11 and B12 were used to demonstrate the quality of the cleaned and original kinase products using oligo 2.

When the ratio of DNA bound at the top of a lane to the amount of DNA in the gel from reaction A is plotted,

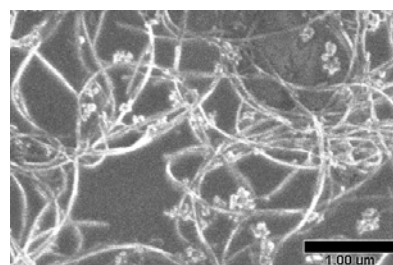


Figure 5. Lambda-DNA clusters on SWNT bundles.

we should see the exponentially decreasing dilution series. Figure 3 plots this ratio as calculated from the gel image in figure 2. The general trend from A1 to A11 is clearly decreasing. The smeared-out background signal seen only in lanes A1–A9 indicates that the reaction conditions widely alter the mobility of the DNA strands. This may be caused by unwanted reactions of primary amines on the A, G, and C nucleotides of oligo 1 with the phospho-diester backbones of other strands. The many amino-groups and phosphate groups available for this reaction could explain the wide variation in the mobility of the products.

4. Conclusions

We have shown that SWNT material can be functionalized with modified DNA. This form of chemical modification is the first step toward implementing a DNA-guided self-assembling process capable of directing the placement of SWNTs. The work presented here in concert with work performed by other groups in DNA metallization [8] and self-assembly [1, 9] presents the start of a compelling argument for the feasibility of self-assembled molecular-scale electronic systems.

References

- [1] Mbindyo J K N, Reiss B D, Martin B R, Keating C D, Natan M J and Mallouk T E 2001 DNA-directed assembly of gold nanowires on complementary surfaces *Adv. Mater.* **13** 249–54

- [2] Liu J, Rinzler A G, Dai H, Hafner J H, Bradley R K, Boul P J, Lu A, Iverson T, Shelimov K, Huffman C B, Rodriguez-Macias F, Shon Y, Lee T R, Colbert D T and Smalley R E 1998 *Science* **280** 1253–6
- [3] Guo Z J, Sadler P J and Tsang S C 1998 Immobilization and visualization of DNA and proteins on carbon nanotubes *Adv. Mater.* **10** 701–3
- [4] Buzaneva E, Karlash A, Yakovkin K, Shtogun Y, Putselyk S, Zherebetskiy D, Gorchinskiy A, Popova G, Prilutska S, Matyshevskaya O, Prilutskyy Y, Lytvyn P, Scharff P and Eklund P 2002 DNA nanotechnology of carbon nanotube cells: physico-chemical models of self-organization and properties *Mater. Sci. Eng. C* **19** 41
- [5] Batalia M A, Protozanova E, Macgregor R B and Erie D A 2002 Self-assembly of frayed wires and frayed-wire networks: nanoconstruction with multistranded DNA *Nano Lett.* **2** 269–74
- [6] Hendrickson J B, Cram D J and Hammond G S 1970 *Organic Chemistry* vol 1, 3rd edn, ed Z Z Hugus (New York: McGraw-Hill) ch 12 pp 468–71
- [7] Wong S S, Woolley A T, Joselevich E, Cheung C L and Lieber C M 1998 *J. Am. Chem. Soc.* **120** 8557–8
- [8] Richter J, Mertig M and Pompe W 2001 *Appl. Phys. Lett.* **78** 536–8
- [9] Gracias D H, Tien J, Breen T L, Hsu C and Whitesides G M 2000 *Science* **289** 1170–2