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In Vitro Transcription and Protein Translation from Carbon Nanotube–DNA Assemblies**

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Single-walled carbon nanotubes (SWNTs), with diameters in the range of 1–2 nm and lengths of a few micrometers, possess very high surface-area-to-volume ratios and offer great potential as advanced multifunctional materials.^[1] While carbon nanotubes have been traditionally investigated for their structural and electrical properties, they are gaining significant attention in biological applications. In particular, the interaction of carbon nanotubes with DNA presents an especially interesting prospect, with potential applications ranging from the dispersion and sorting of nanotubes^[2] to biomolecular sensing.^[3–7] Consequently, a variety of covalent techniques, including amidation,^[3,4] reduction of nitro groups,^[5] and cleavable disulfides,^[6] have been employed for the immobilization of DNA to SWNTs. Non-covalent attachment of DNA to SWNT surfaces, mediated primarily via electrostatic interactions, has also been employed with equal success.^[7–9]

While these approaches have resulted in SWNT–DNA conjugates, few have exploited the intrinsic biological function of the DNA, although cellular delivery and gene expression has been observed.^[10] In the current work, we describe the generation of assemblies consisting of DNA adsorbed onto SWNTs by means of electrostatic interactions^[11] and subsequently demonstrate that these hybrids can be used as templates for RNA polymerase-catalyzed gene transcription, which can then lead to in vitro protein synthesis. This is the first report of gene transcription from SWNT–DNA conjugates, and, therefore, this work may have significant implications for the development of high-throughput protein synthesis systems with ultimate applications in functional genomics.

Our strategy involved the noncovalent functionalization of SWNTs with cationic moieties that would strongly bind to the negatively charged phosphate backbone of the DNA. To that end, a SWNT bucky paper (Figure 1a) was initially

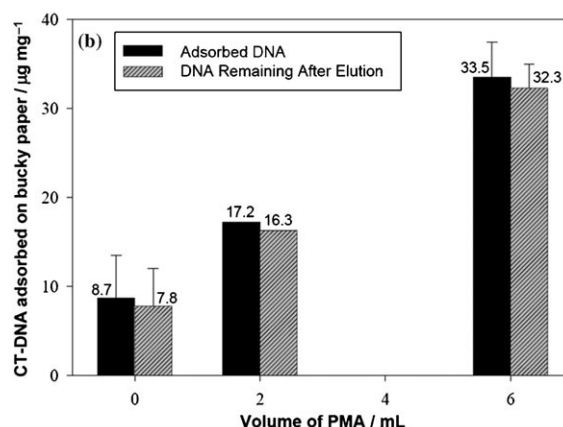
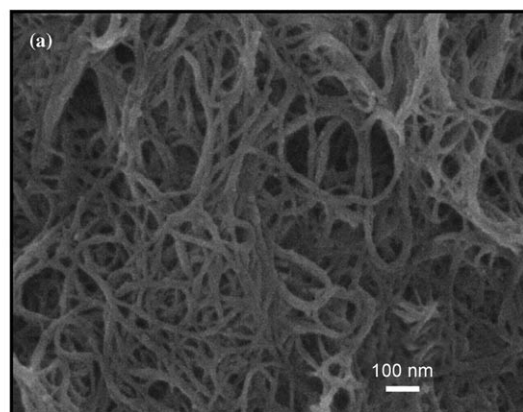


Figure 1. a) SEM image of a SWNT bucky paper. b) Effect of added volume of 1-pyrenemethylamine hydrochloride (PMA) on the amount of calf-thymus DNA (CT-DNA) adsorbed per mg of the SWNT bucky paper. The volume of PMA (10 mM in methanol) was varied from 0 to 6 mL (0–60 µmol PMA) and 0.7 mg of SWNTs were used. The adsorbed DNA was first washed twice with buffer (50 mM Tris buffer, pH 7.0) followed by two washes with buffer-containing salt (50 mM Tris buffer, pH 7.4, plus 0.6 M NaCl) to yield the DNA that remained adsorbed following washing. The loading of CT-DNA adsorbed on the bucky paper was calculated by a mass balance.

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treated with 1-pyrenemethylamine hydrochloride (PMA) and then incubated with calf-thymus DNA (CT-DNA), which represents a heterogeneous mixture of DNA. Such a mixture was deemed to be a good initial model to demonstrate DNA–SWNT interactions as it would represent a range of DNA sequences and would not be specific to any one sequence of ultimate interest. The amount of DNA adsorbed on SWNT was calculated by the mass balance of the initial DNA and the amount left in the supernatant after equilibration.

Figure 1b shows the mass of CT-DNA adsorbed onto the SWNT normalized by the weight of the bucky paper as a function of the volume of PMA (0, 2, and 6 mL) employed

in the loading experiments. CT-DNA ($\approx 6\%$ of that added to solution) was adsorbed onto the SWNT even in absence of PMA, presumably due to nonspecific hydrophobic interactions or physical entanglements. However, increasing the volume of PMA added increased the amount of CT-DNA adsorbed onto the SWNT, such that at 6 mL of PMA, $\approx 25\%$ of the CT-DNA added to solution bound to the SWNT. This increase in binding may be attributed to the electrostatic attraction between the negatively charged phosphate groups of the DNA backbone and the positively charged amino groups on the PMA. The stability of the SWNT–DNA hybrid was tested by washing with 600 mM NaCl and analyzing the wash supernatants for free DNA via complexation with ethidium bromide. As seen in Figure 1b, negligible amounts of CT-DNA desorbed from the SWNT, indicating that the adsorption led to stable attachment.

To directly image the nanotube-bound CT-DNA, we extended this approach to the adsorption of CT-DNA onto individual SWNTs and small bundles supported on silicon dioxide substrates. Figure 2 depicts AFM images of the CT-

DNA occurs along the axis of the nanotubes and that electrostatic interactions are the predominant driving force for CT-DNA adsorption. Furthermore, the contrast between the pristine portion of the nanotubes and the thick regions in the AFM phase image (Figure 2b) indicates that the adsorbed species is molecularly different from the nanotubes. Finally, there is no evidence of DNA chains (in either AFM or SEM) partially desorbed from the SWNT, for example, as loops or segments that do not lie along the axis of the SWNT. Such partial DNA attachment is unlikely given the high complementary charge densities of both the PMA and the phosphodiester backbone of the DNA, which strongly favors full-length DNA adsorption.

The adsorption of DNA onto SWNTs, free or in bucky paper form, does not provide direct evidence that the DNA is functional. One of the more demanding roles of DNA, and indeed its most important functional property, is its ability to be transcribed by RNA polymerase thereby leading to the synthesis of mRNA and ultimately protein synthesis, which has also been done under *in vitro* conditions.^[12,13] For transcription to occur, the DNA must retain its native structure and be accessible to the RNA polymerase. In previous work, we demonstrated that DNA encoding for luciferase could be transcribed when attached to streptavidin (via a biotin linker) covalently attached to the wells of a 96-well plate.^[14] In that case, the DNA was attached only through its 3' end. The resulting mRNA was then coupled to an *in vitro* translation system for the synthesis of the enzyme luciferase. The negligible luminescence background in the absence of functional luciferase makes this system ideal for the current study. We reasoned that a similar *in vitro* synthesis of luciferase encoded by a luciferase plasmid adsorbed onto the SWNTs would provide a definitive indication that the bound DNA retains its native biological activity upon adsorption, even though the DNA is bound flat on the axis of the SWNT.

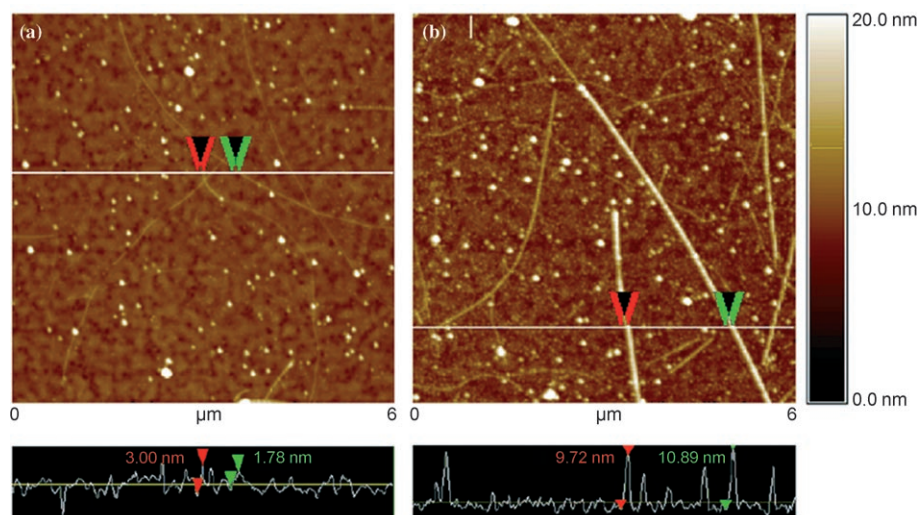


Figure 2. AFM topography images and height profiles (vertical axis not to scale) of calf-thymus DNA (CT-DNA) adsorbed on substrate-supported SWNTs: a) Direct adsorption of CT-DNA. b) The substrate was first equilibrated with 6 mL PMA and then with CT-DNA. The thickening of the nanotubes in (b) indicates adsorption of the DNA on the nanotube surface. The SWNT–DNA conjugates in Figure 2b are somewhat variable, providing some evidence that DNA aggregates may form, although if such aggregates do form they are no larger than two DNA strands based on the diameter of DNA and the thickness of the SWNT–DNA conjugates.

DNA adsorbed on the SWNTs. A negligible increase in thickness of the SWNTs was observed with the direct adsorption of CT-DNA in the absence of PMA (Figure 2a), and individual or at most double SWNT filaments were obtained. Conversely, pre-equilibration of the SWNTs with PMA followed by incubation with CT-DNA led to a clearly visible thickening (≈ 10 nm) of the nanotubes along their length (Figure 2b). This size is consistent with either two DNA chains attached to the SWNT or one chain wrapping around the SWNT. Scanning electron microscopy (SEM, not shown) did not reveal large-scale aggregation of the surface-bound SWNT bundles, suggesting that adsorption of CT-

We initially adsorbed the luciferase plasmid onto SWNT bucky paper (0.7 mg) treated with 6 mL PMA, as was done above with CT-DNA. This plasmid contains the T7 promoter sequence required for *in vitro* transcription and is also linear, thereby guaranteeing that the full gene would be contact with the SWNTs. After adsorption, the supernatant was removed (wash 1) and the SWNT–DNA assemblies were washed an additional four times with 50 μ L Tris buffer (pH 7.4); the duration of each wash lasted 30 min. The loading of the DNA on the bucky paper was measured to be 1.85 μ g mg⁻¹ bucky paper by mass balance. This was far lower than CT-DNA (Figure 1b), which was likely due to

the lower amount of luciferase DNA that was added to the loading solution. Specifically, the efficiency of binding for CT-DNA was nearly one quarter, that is, $\approx 32 \mu\text{g}$ CT-DNA per mg of SWNT bound versus $\approx 128 \mu\text{g}$ CT-DNA per mg of SWNT challenged. For luciferase DNA, the amount bound was $1.85 \mu\text{gmg}^{-1}$ SWNT, while $\approx 7.14 \mu\text{gmg}^{-1}$ SWNT was challenged. Thus, the fraction of challenged DNA that adsorbed onto the SWNT bucky paper was similar for both CT-DNA and luciferase DNA, which indicates, as expected, that the SWNT does not discriminate between two very different DNA sequences. The washes were then analyzed for the total amount of luciferase produced using a wheat germ extract *in vitro* coupled transcription–translation (TNT) system. All adsorption experiments and washes with the SWNT–luciferase plasmid assemblies were conducted at 8°C . As shown in Figure 3, a significant amount of the luci-

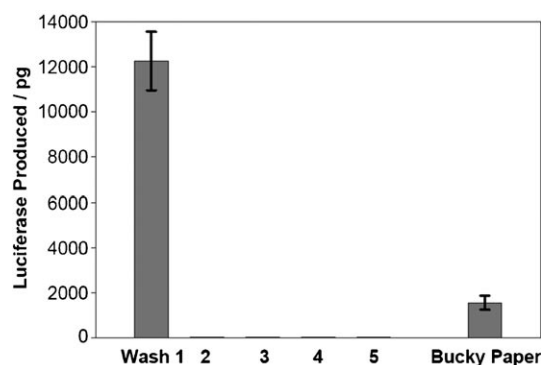


Figure 3. *In vitro* TNT results on the T7 linear control plasmid immobilized on a SWNT bucky paper; vertical bars represent the total amount of luciferase produced. After adsorption of the plasmid on the bucky paper, the assembly was washed five times (see text for details) and each wash was assayed for luciferase production. Each wash was carried out for 30 min at 8°C . In addition, the TNT assay was also performed on the bucky paper. Luciferase synthesis on the bucky paper even after extensive washing is an indication that a fraction of the plasmid remains bound and the DNA remains functional on the nanotube surface.

ferase was produced from the first wash, which consisted solely of the supernatant from the loading step. This result is consistent with the majority of luciferase DNA challenged onto the SWNTs remaining unbound. Subsequent washes resulted in negligible luciferase synthesis.

Having established that most of the loosely bound luciferase plasmid was desorbed in the initial supernatant, and that negligible luciferase DNA was removed in subsequent washes, we performed the *in vitro* transcription and translation assay on the plasmid stably adsorbed onto the SWNT bucky paper recovered after the fifth wash (last entry in Figure 3). Active luciferase was synthesized from the SWNT–DNA assembly, indicating that the bound DNA was functional toward RNA polymerase-catalyzed transcription. The specific activity of the unbound luciferase DNA was calculated from the luciferase generated divided by the amount of DNA in the supernatant. This led to a value of 3.3 ng luciferase per μg DNA. The bound DNA after the ex-

tensive washing had a specific activity of $1.2 \text{ luciferase } \mu\text{g}^{-1}$ DNA. Thus, $\approx 36\%$ of the bound luciferase DNA remained functional as compared to the native solution DNA in coupled transcription/translation reactions.

To confirm that the SWNT–DNA assemblies remained intact during transcription and that the bound DNA was not released from the SWNT due to the action of the RNA polymerase, wherein effectively solution-phase transcription would ensue, we washed the SWNT–DNA complex extensively with buffer containing RNA polymerase and measured the transcription/translation activity of the wash solution and the remaining SWNT–DNA assemblies. Specifically, the SWNT–PMA complex was incubated at 8°C for 18 h with luciferase DNA solution as before. The supernatant was then removed and the SWNT–DNA was washed six times with 1 mL Tris buffer (20 mM, pH 7.4) and six times with Tris buffer containing $1 \mu\text{L}$ of RNA polymerase without the nucleotide triphosphates required for mRNA synthesis.

As seen in Figure 4, the luciferase gene remaining on the SWNT was successfully transcribed to functional lucifer-

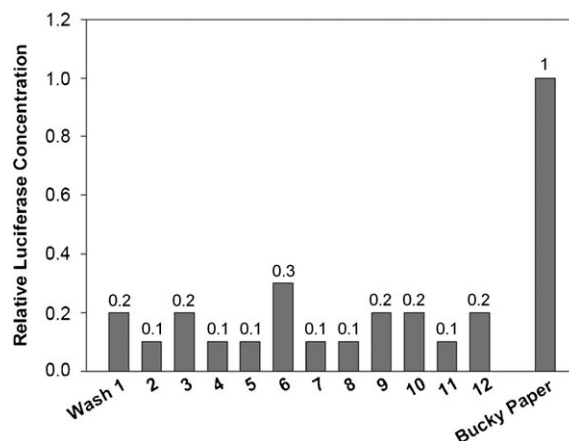


Figure 4. Effect of RNA polymerase washes on luciferase production from SWNT–DNA assemblies. The bucky paper with adsorbed plasmid was washed first with 20 mM Tris buffer (washes 1–6) and then with 20 mM Tris buffer plus $1 \mu\text{L}$ RNA polymerase (20 units mL^{-1} ; washes 7–12). Most of the initially unbound (or loosely bound) DNA was removed in the initial loading of the supernatant (not shown in this figure). The washes were assayed for luciferase activity, following which the TNT assay was carried out directly on the bucky paper. The luciferase concentration for the washes was normalized with respect to the bucky paper for comparison.

ase even after 12 washes, including six with RNA polymerase. The addition of RNA polymerase as a control was based on the possibility that this enzyme could remove the DNA from the SWNT surface and into solution where solution-phase transcription could result. However, our results clearly indicate that the luciferase gene was not removed during the catalytic action of RNA polymerase and that transcription occurs directly on the surface of the SWNT. We may envision that such a biological process occurs as a result of the RNA polymerase interacting directly with the

bound linear DNA, where it recognizes the T7 promoter sequence on the DNA to initiate transcription on one of the coding strands of the DNA. In the process, the DNA strands are transiently separated (melted) to enable transcription of the DNA template into mRNA. A schematic of this process proposed to occur on the SWNT is depicted in Figure 5, which shows the RNA polymerase melting a segment of the bound double-stranded DNA, thereby leading to mRNA synthesis that leads ultimately to luciferase synthesis.

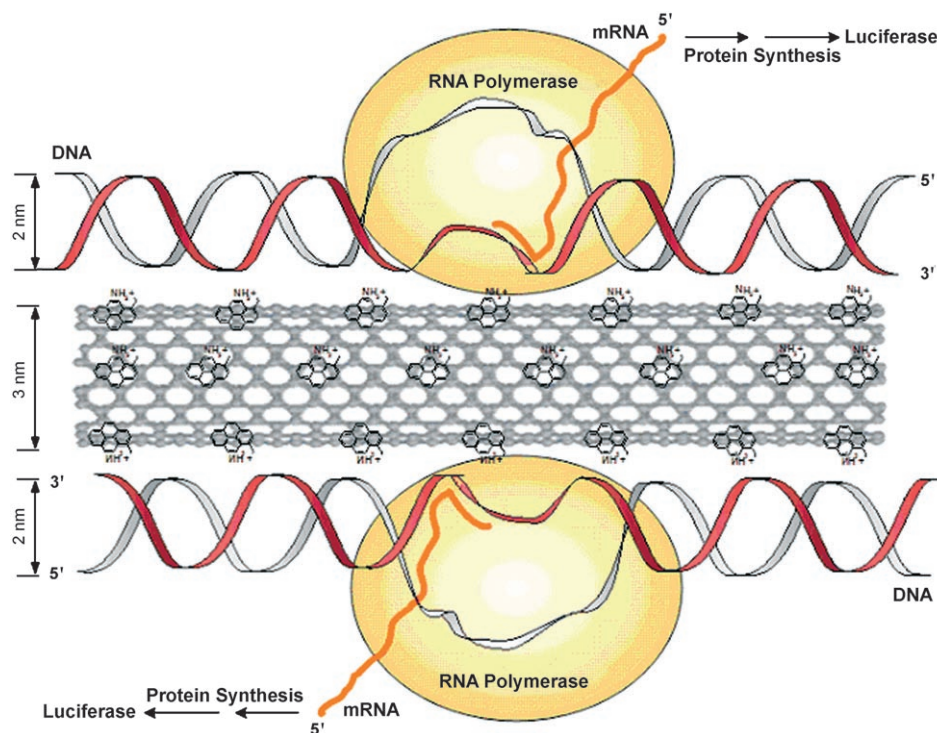


Figure 5. Cartoon schematic of the proposed action of RNA polymerase in vitro on SWNT–DNA conjugates. The size of the conjugates is given along with the approximate size of the RNA polymerase. An overall thickness of ≈ 7 nm is expected for the SWNT–DNA conjugates. The presence of an aggregate of two DNA molecules on some fraction of the surface would add 2–4 nm in additional thickness.

In conclusion, we have demonstrated that functional genetic material can be adsorbed onto SWNTs. The ability of SWNT–DNA assemblies to retain the intrinsic functional properties of DNA indicates that the nanoscale DNA constructs remain structurally intact, and can further participate in complex biological events. This is a fundamental requirement for applications ranging from sensitive hybridization-based sensors to building blocks for the assembly of supra-molecular structures.

Experimental Section

Purified SWNTs produced by the HiPCO process were obtained from Carbon Nanotechnologies Inc. (Houston, TX). SWNTs (20 mg) were bath-sonicated in methanol (150 mL) for 15 min. The dispersion was then vacuum-filtered over a polycarbonate membrane filter (0.2 μm pore size). The resulting mat of SWNTs that deposited onto the filter was dried at 50 °C in vacuum over-

night. The dried nanotube mat was easily peeled off from the filter to yield the bucky paper.

Well-dispersed, low-density SWNTs were grown on thermal silicon dioxide substrates using a CVD growth process. Iron catalyst was evaporated on the substrate before growth and methane was used as the carbon source. Growth was carried out at 900 °C and 500 Torr. AFM was carried out in the tapping mode using a Multimode Nanoscope IIIa microscope from Digital Instruments Inc (Santa Barbara, CA).

The preparation of DNA–carbon nanotube assemblies was as follows: a SWNT bucky paper (20 mg) was initially treated with different volumes of 1-pyrene-methylamine hydrochloride (PMA) (10 mM) in methanol at 25 °C for 3 h with shaking at 75 rpm. After incubation, the SWNT bucky paper–PMA complex was washed twice with methanol (2 mL) and three times with Tris buffer (2 mL, 20 mM, pH 7.4). After air drying, calf-thymus DNA (CT-DNA; 15 mL, 6 $\mu\text{g mL}^{-1}$) was equilibrated with the bucky paper–PMA complex (0.7 mg) for 18 h at 25 °C. After equilibration, the supernatant was removed and complexed with ethidium bromide to determine the concentration of the CT-DNA in the supernatant. The loading of CT-DNA adsorbed on the bucky paper was calculated by a mass balance. A similar procedure was applied to linearized luciferase DNA (0.1 mL with a concentration of 50 $\mu\text{g mL}^{-1}$) and the incubation was performed at 8 °C. For visualization of CT-DNA on individual SWNTs, we used a similar procedure as that described for adsorption to the bucky paper.

An in vitro coupled transcription–translation reaction (TNT) was performed according to standard protocols using the Promega Wheat Germ Extract TNT T7 kit (www.promega.com). The following approach was used: to buffer (2 μL), RNasin (ribonuclease inhibitor; 1 μL), WG T7 RNA polymerase (1 μL), amino acid mixture (complete; 1 μL , 1 mM), DEPC-treated water (10 μL), and TNT T7 wheat germ extract (25 μL) were added. The contents were mixed on ice and introduced onto the bucky paper containing the luciferase DNA (for an estimated total volume of ≈ 50 μL). The tube was then immediately incubated at 30 °C for 3 h. After incubation, the mixture (2.5 μL) was applied to Steady Glo Luciferase Assay Reagent (Promega; 50 μL). This was repeated four times (using a total of 10 μL of the 50 μL reaction mixture) for statistical analysis, in a white Microfluor 96-well plate. The plate was then read in luminescence mode at a gain of 150 and a constant integration time of 1 s. The luminescence (Relative Luminescence Units, RLU) measured is directly proportional to the amount of the luciferase produced ($\text{pg } \mu\text{L}^{-1}$).

Keywords:

carbon nanotubes • catalysis • DNA • enzymes •
gene transcription

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