Carbon Nanotubes Protect DNA Strands during Cellular Delivery

Yanrong Wu,[†] Joseph A. Phillips,[†] Haipeng Liu,[†] Ronghua Yang,[‡] and Weihong Tan*,[†]

[†]Center for Research at Bio/nano Interface, Department of Chemistry and Department of Physiology and Functional Genomics, Shands Cancer Center, UF Genetics Institute and McKnight Brain Institute, University of Florida, Gainesville, Florida 32611-7200, and [‡]Biomedical Engineering Center, State Key Lab of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, China

ABSTRACT To protect against nuclease digestion, or single-strand binding protein interactions, oligonucleotides for targeted delivery into intracellular systems must be stable. To accomplish this, we have developed single-walled carbon nanotubes as a carrier for single-stranded DNA probe delivery. This has resulted in superior biostability for intracellular application and, hence, has achieved the desired protective attributes, which are particularly important when DNA probes are used for intracellular measurements. Specifically, when bound to single-walled carbon nanotubes, DNA probes are protected from enzymatic cleavage and interference from nucleic acid binding proteins. Moreover, and equally important, our study shows that a single-walled carbon nanotube-modified DNA probe, which targets a specific mRNA inside living cells, has increased self-delivery capability and intracellular biostability when compared to free DNA probes. Therefore, this new conjugate provides significant advantages for basic genomic studies in which DNA probes are used to monitor intracellular levels of molecules.

KEYWORDS: carbon nanotubes · DNA · protection · cell · delivery · molecular beacon

Biomedical applications, particularly those which involve targeted drug delivery, depend upon uninhibited transport of DNA, RNA, or drug molecules into living cells. However, some cargos, such as DNA, are easily degraded by cellular enzymes or digested by cellular nucleases. This problem is compounded by the fact that most delivery systems take several hours to transport cargos into cells. Therefore, a delivery system which can provide protection for DNA cargos during prolonged transport would be useful. To this end, inorganic nanomaterials, including nanoparticles, nanotubes, and nanowires, have exhibited promising physical properties which make them useful as molecular transporters.[1−10] To date, however, only a few nanomaterials, such as silica nanoparticles,[2] silica nanotubes,[8] and gold nanoparticles,[11] offer viable protection properties. Aside from these, the most promising of all may be single-walled carbon nanotubes (SWNTs) which have been shown to shuttle various types of cargos into a wide range of cell types. These include the biologically and medically more relevant T cells and primary cells, which are difficult to transfect by traditional delivery methods.[12,13] Therefore, this research investigates whether SWNTs can, additionally, provide protective properties similar to silica or gold nanoparticles, thus ultimately shielding bound DNA sequences from cleavage during *in vivo* cellular delivery.

RESULTS AND DISCUSSION

To investigate the protective properties of SWNTs, a 30-base-paired single-stranded DNA (ssDNA) oligonucleotide with repeating G−T sequence (GT) was used as the model sequence. This sequence has been demonstrated to wrap onto the SWNT surface.[14,16] Radioisotopic labeling and denaturing PAGE gel were then used to monitor the digestion of DNA by DNase I, which can nonspecifically cleave ssDNA and dsDNA. The efficacy of the method was first tested to determine whether SWNTs could affect the mobility of bound DNA. To accomplish this, both GT/SWNT complex and free DNA were treated with DNase I, and aliquots were collected at 5, 15, and 60 min time points. Aliquots were heated at 95 °C for 5 min before running the denaturing PAGE gel. As shown in Figure 1, the mobility of both GT and GT/SWNT sequences remains the same (lanes 1 and 2), which demonstrates that SWNTs do not inherently affect the mobility of their bound DNA. Having determined the viability of the method for monitoring digestion in the GT/SWNT complex cases, the same model and protocol were used to determine the effect of cleavage. Accordingly, the results of the cleavage assay show increased digestion of GT as a function of time, but no digestion for GT/SWNT, even after 60 min of digestion

*Address correspondence to tan@chem.ufl.edu.

Received for review May 28, 2008 and accepted September 19, 2008.

Published online October 8, 2008.
10.1021/nn800325a CCC: $40.75

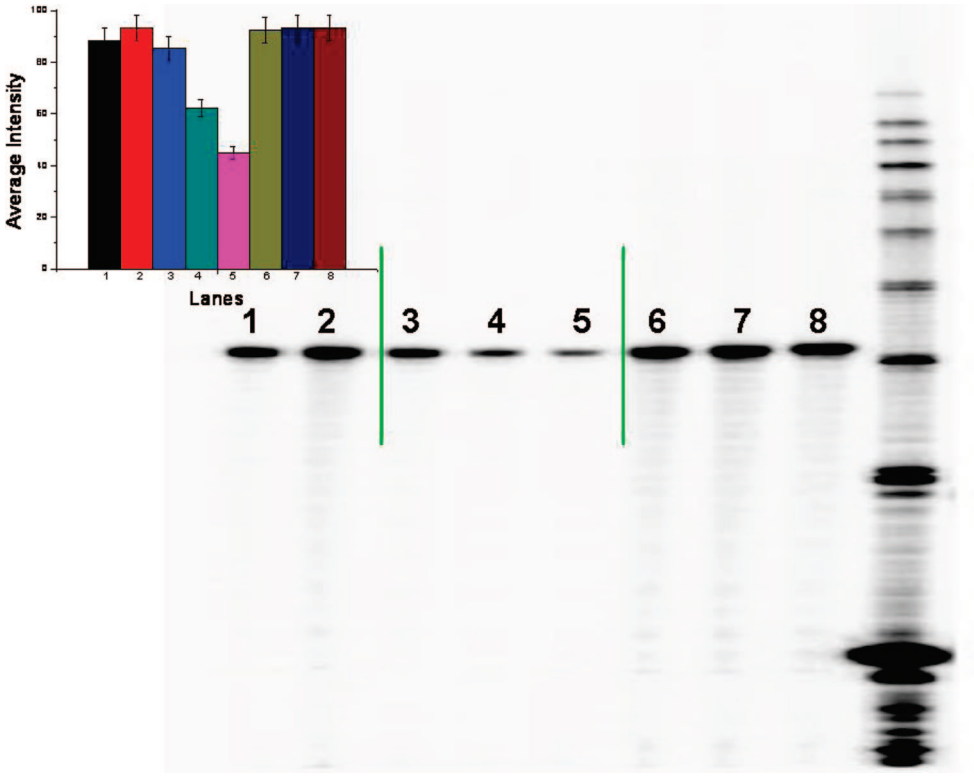
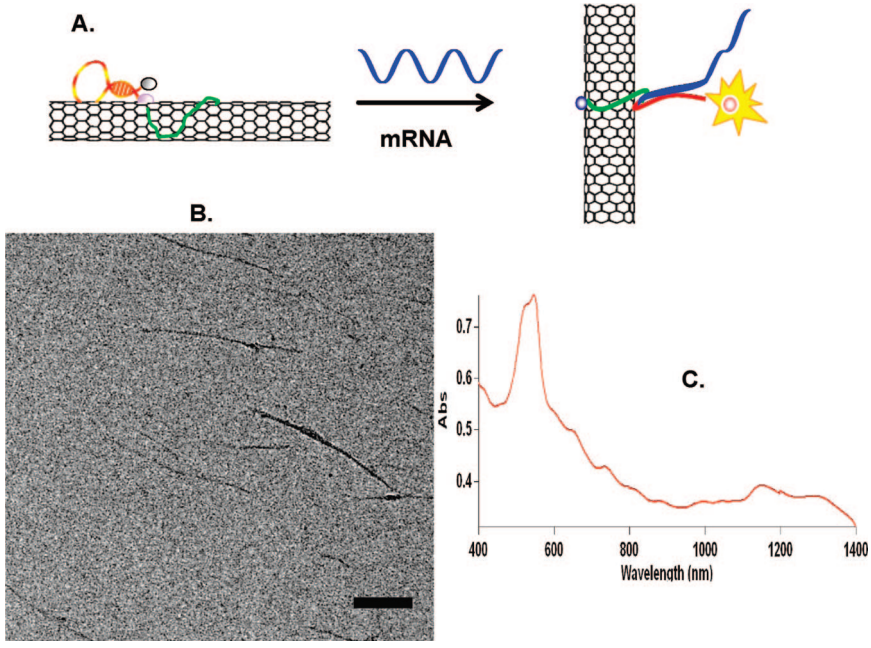


Figure 1. Polyacrylamide gel electrophoresis (PAGE) of free GT sequence and GT/SWNT complexes by 15% gel. Lanes 1 and 2 are the intact GT sequence and GT/SWNT complexes; lanes 3−5 are the GT sequence after DNase I digestion for 5 min, 15 min, and 1 h; lanes 6−8 are the GT/SWNT complexes after DNase I digestion for 5 min, 15 min, and 1 h; lane 9 is the 10 base pair DNA marker. The gel band intensity for each lane is plotted in the upper left corner graph.



Scheme 1. (A) Possible interaction between MnSOD probes, the SWNTs, and target mRNA; (B) TEM image of MnSOD probe/SWNTs (the scale bar represents 100 nm); (C) absorption spectrum of the probe in H_2O.

(Figure 1, lanes 3−5 and lanes 6−8, respectively). These experiments demonstrate that GT DNA is protected from DNase I cleavage when it is in complex with the SWNTs. Although protection from enzymatic cleavage is a useful property for DNA delivery into cells, it is also important that DNA probes be functional when in complex with SWNTs.

Therefore, in order to demonstrate that SWNTs can protect functional DNA probes from enzymatic cleavage, a specific DNA probe was first modified and complexed with the SWNTs (possible interaction is shown in Scheme 1A). This DNA probe, which showed increased fluorescence upon binding manganese superoxide dismutase (MnSOD) mRNA,[15] was further modified with polyT[16] to increase binding to the SWNT. In the absence of target cDNA (cDNA), our previous study[17] demonstrated that the designed probe forms a hairpin structure and adsorbs onto the SWNTs at room temperature, resulting in low signal intensity from the MnSOD probe/SWNTs. This low signal intensity was mainly a consequence of quenching from the Black Hole Quencher 2 (BHQ2), but it could have also been the result of quenching from the SWNTs.[18] On the other hand, in the presence of target cDNA, the hybridization event separated the fluorophore from the quencher, or SWNTs, thus causing the signal enhancement shown in Figure 2. This demonstrates that the MnSOD probe/SWNTs could still respond to the target cDNA and that, consequently, the bound DNA was still functional.

Since the functional sensitivity of the MnSOD probe/SWNTs could be retained, we next tested its ability to resist nuclease cleavage *in vitro*. First, a fluorescence-based assay was performed as follows. One unit of DNase I endonuclease was added to separate solutions of 50 nM free MnSOD probes and probe/SWNTs. In this assay, if the probe is digested by DNase I, the fluorescence intensity will increase because the

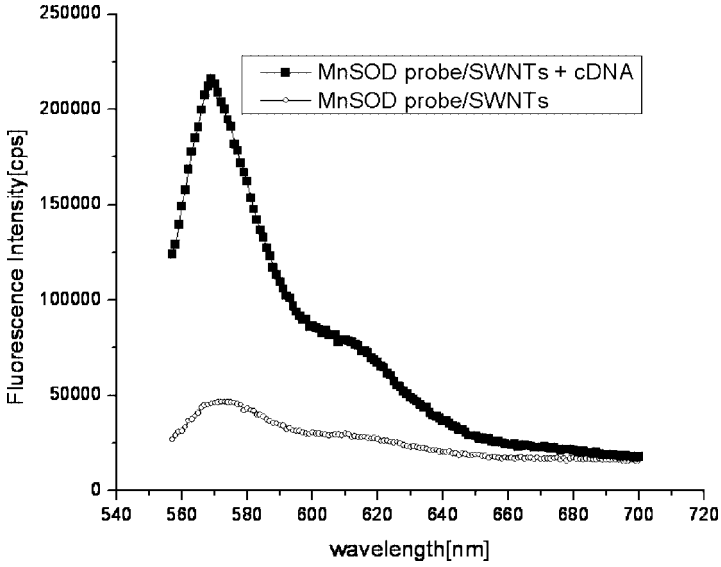


Figure 2. Emission fluorescence spectrum of MnSOD probe/SWNTs with and without the presence of 10-fold excess target cDNA.

donor dye molecule will separate from the quenchers. Experiments for both free MnSOD probes and MnSOD probe/SWNTs were performed under identical conditions. As shown in Figure 3, probe/SWNTs showed little degradation and, correspondingly, little fluorescence, while the free MnSOD probes showed a significant fluorescence increase, indicating increased degradation. These preliminary results therefore demonstrate that MnSOD probe/SWNTs could be immune to cleavage by DNase I. Next, the interactions of free MnSOD probes and probe/SWNTs with single-stranded DNA binding protein (SSB) were investigated. This was necessary because, intracellularly, DNA probes are subject to nonspecific binding by proteins, which can produce false positive signals. One such protein is the ubiquitous single-stranded DNA binding protein.[19] As shown in Figure 3, free probes had a 6-fold increase of fluorescent signal compared to MnSOD probe/SWNTs when incubated with SSB. Since the probe/SWNT complex showed little response to excess SSB, these results demonstrate that it may well be protected from this form of interference during intracellular experiments.

In the context of their promising DNA protective properties, as demonstrated by these two *in vitro* experiments, probe/SWNT complexes show improved biostability when compared to the free probe. However, to further confirm these protection properties, the natural ability of SWNTs to be internalized was used to test the probe/SWNTs in a cellular environment. In this assay, the probe/SWNTs are delivered by simply incubating the complexes with MDA-MB-231 breast carcinoma cells. Under normal culture conditions, this cell line has a low MnSOD expression level; however, when exposed to lipopolysaccharide (LPS), an inflammatory mediator involved in *Escherichia coli* bacterial sepsis, MnSOD mRNA expression levels increase substantially.[15,20] As a result of the resistance of probe/SWNTs to enzymatic cleavage and nonspecific opening, the probe/SWNTs complexes should show lower fluorescence background compared to the free probes before the cells are stimu-

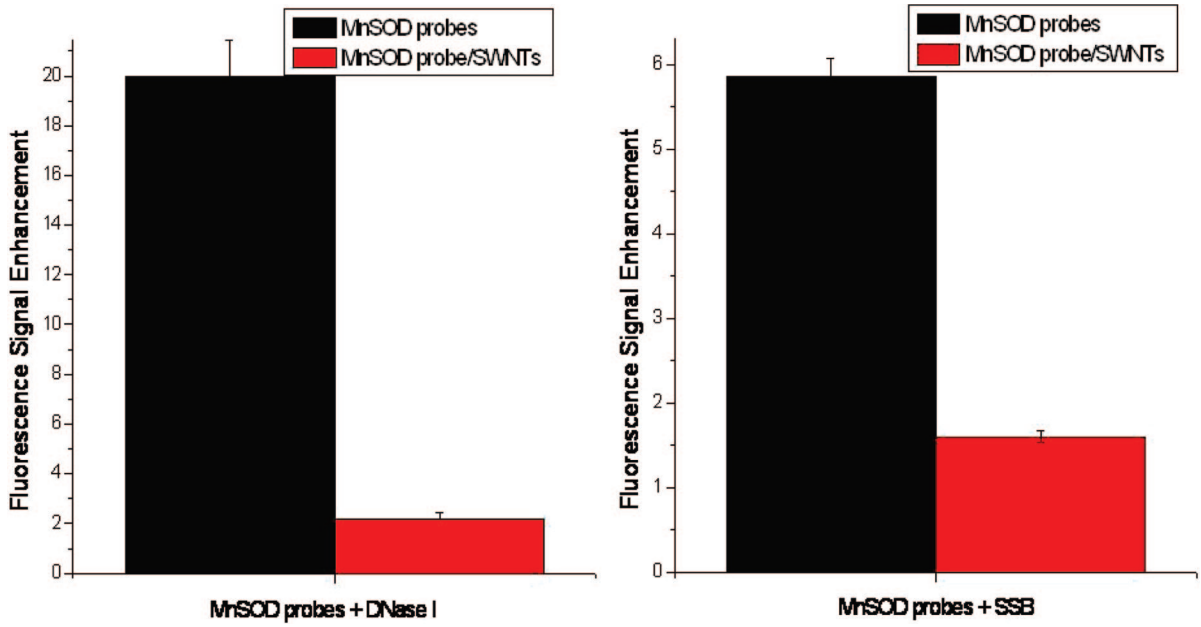


Figure 3. (Left) Fluorescence signal enhancements of both free MnSOD probes and MnSOD probe/SWNTs upon the addition of 1U DNase I. (Right) Fluorescence signal enhancements of both probes upon the addition of SSB. Final concentration ratio of probe/SSB = 1:5.

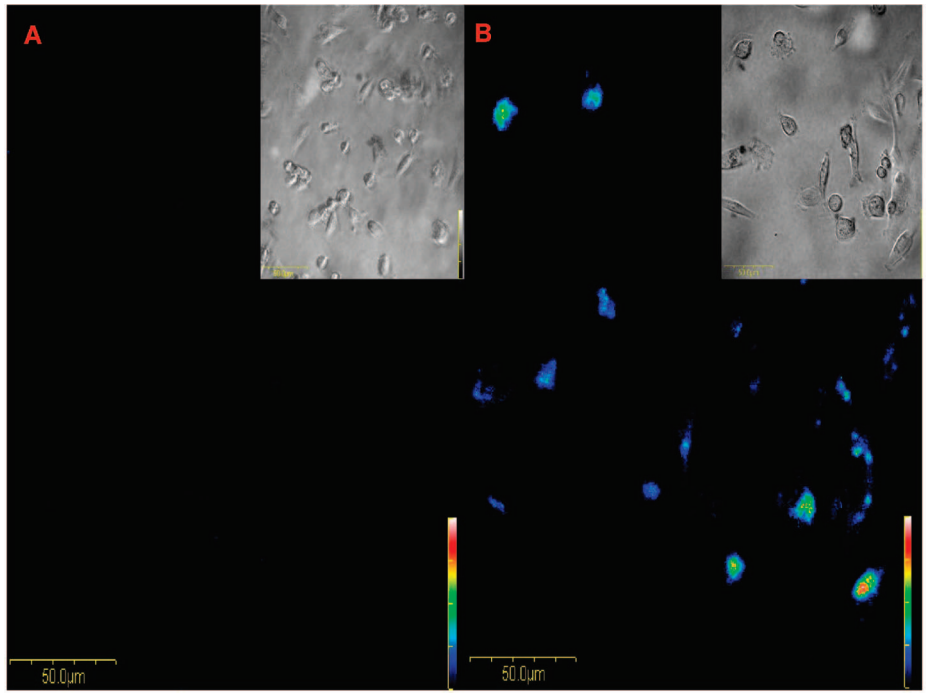


Figure 4. Bright field and fluorescent images of MnSOD probe/SWNTs inside the MDA-MB-231 cells, (A) without LPS stimulation and (B) with LPS stimulation. All the scale bars shown in the graph are 50 μm.

lated with LPS. Following LPS stimulation, if the probe/SWNTs are still functional after long intracellular incubation times, hybridization with target mRNA sequences will produce a fluorescent signal that can be detected by confocal microscopy.

Briefly, 20 μM probes were mixed with 200 mg/L nanotube aqueous solution, sonicated, and centrifuged. The pellet comprising the nanotube impurities and aggregates was discarded, and the supernatant was collected as the probe/SWNTs, which turned out to be soluble. The probe/SWNTs were then incubated with MDA-MB-231 breast carcinoma cells at a final concentration of about 25 mg/L for 12 h. Subsequently, the cells were washed and treated with or without 1 μg/mL LPS for another 4 h before imaging. As shown in Figure 4, a high fluorescence signal was observed for most of the cells after LPS stimulation, which indicated the up-regulated MnSOD mRNA expression. Control experiments without LPS stimulation were carried out under the same conditions, and little fluorescence was observed compared to the LPS-stimulated cells. This experiment was repeated, and it was further demonstrated that probe/SWNT complexes produce a few false positive signals. For comparison, free MnSOD probes were incubated with cells under the same conditions, and low fluorescence signal was observed with and without LPS stimulation (Figure 5). The background for the free probe without LPS stimulation was higher than that of the complexes without LPS stimulation. This might have been the result of digestion and the nonspecific opening of very few MnSOD probes that were inefficiently self-delivered into the cells after a long incubation time, leading to a false positive signal. It might have also been caused by some probes being trapped between the cells. If these probes had been digested or disrupted, they would have had no ability to detect the different gene expression levels, causing the same fluorescence intensity to be observed with LPS stimulation. To confirm this explanation, another free random DNA probe with no complement inside the cells was tested under the same conditions, and results identical to those of the

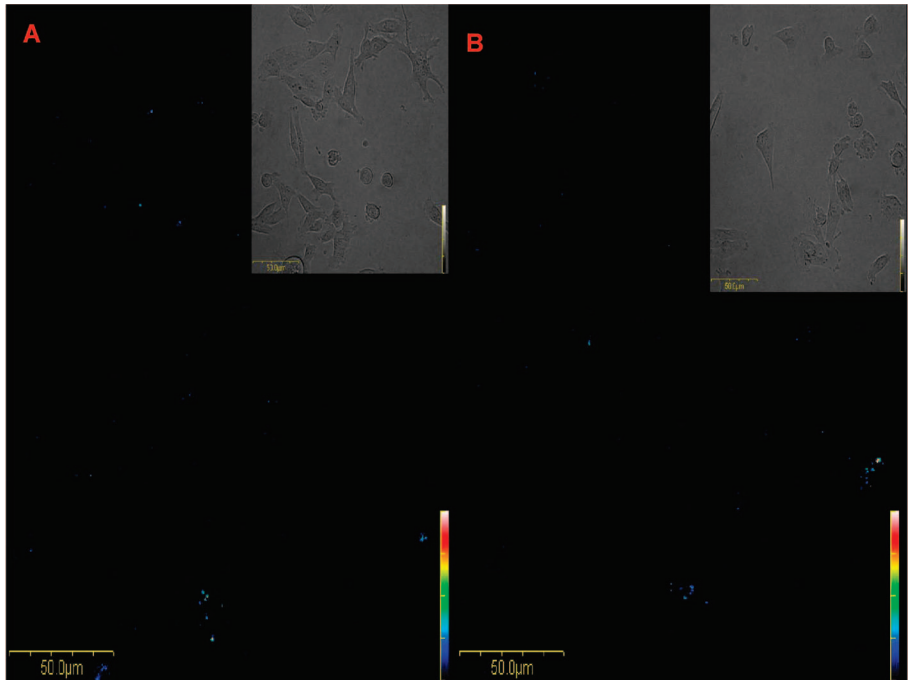


Figure 5. Bright field and fluorescent images of free MnSOD probes inside MDA-MB-231 cells, (A) without LPS stimulation and (B) with LPS stimulation. All the scale bars shown in the graph are 50 μm.

free MnSOD were obtained, as expected. Thus, the single-walled carbon nanotubes do protect probes from digestion and disruption which ensures that the DNA probes can successfully distinguish different gene expression levels free from the types of interference examined in this paper.

These two sets of experiments, both *in vitro* and in cell culture, have demonstrated improved biostability of probe/SWNTs over the free probes. Specifically, the *in vitro* experiments showed that probe/SWNTs have much better resistance to nuclease digestion and nucleic acid binding protein disruption. Furthermore, the intracellular experiments showed that probe/SWNTs complexes are functional, even after a total incubation of 16 h. In contrast, free DNA degrades after only 15−45 min in the cellular environment.[21] As a consequence of reduced nonspecific opening events, lower background improved the detection sensitivity of probe/SWNT complexes. Therefore, this investigation proves that SWNTs have the ability to protect bound DNA cargos from enzymatic cleavage and DNA binding proteins both during and after delivery into cells.

As noted above, different nanomaterials exhibit various degrees of DNA protection, but the mechanisms of protection are not yet well understood. For example, silica nanoparticles exhibit protection of plasmid DNA. It has been proposed that the positive charges on the silica nanoparticle surface can exclude Mg^{2+} and that the DNA conformational change due to binding onto this nanoparticle surface prevents digestion.[2] In the case of silica nanotubes, the authors hypothesized that the nanotubes act as a physical shield that protects the loaded materials from environmental damage.[8] To date, no reasoning has been provided to explain the efficacy of gold nanoparticles.[11] The protective properties of SWNTs, on the other hand, may be explained in several ways. First, the probes could be embedded inside small bundles of nanotubes such that the nucleases/proteins cannot physically access the DNA. Second, although the surface of the SWNT has been modified with hydrophilic groups from the DNA probes, some hydrophobic regions may still remain exposed and cause inhibitory effects on proteins that come into close proximity. Finally, the interaction between DNA and SWNTs[16] may cause the secondary structure of the DNA to be unrecognizable to enzyme binding pockets. Obviously, further investigation is required to address these causal issues to conclusively determine the mechanisms underlying the protective properties of probe/SWNTs complexes.

CONCLUSIONS

In summary, when bound to SWNTs, DNA probes are protected from enzymatic cleavage and interference from nucleic acid binding proteins. These protective properties are particularly important for applications in which DNA probes are used for intracellular measurements. Our study shows that a SWNT-modified DNA probe, which targets a specific mRNA inside living cells, has increased self-delivery capability and intracellular biostability when compared to free DNA probes. Therefore, this novel material provides significant advantages for basic genomic studies in which DNA probes are used to monitor intracellular levels of molecules and ions. Additionally, for cytoplasmic gene detection by DNA probes, nuclear sequestration is a major cause of reduced sensitivity.[22] The DNA/SWNT complexes, however, stay within the cytoplasm, enabling cytoplasmic mRNAs to be detected and imaged. Furthermore, DNA/SWNT complexes should prove useful as therapeutic agents since they exhibit excellent self-delivery properties that could allow DNA-based drugs to exert their therapeutic presence for longer time before being degraded by cells.

EXPERIMENTAL METHODS

Materials and Instruments. The sequences of DNA and RNA oligonucleotides prepared are listed in Table 1. DNA synthesis reagents were purchased from Glen Research (Sterling, VA). The SWNTs were purchased from Unidym, Inc. with <5 wt % ash content (CAS number: 7782-42-5). An ABI3400 DNA/RNA synthesizer (Applied Biosystmes, Foster City, CA) was used for all MB probes and DNA target preparation. A ProStar HPLC (Varian, Walnut Creek, CA) with a C18 column (Econosil, 5 μm, 250 × 4.6 mm) from Alltech (Deerfield, IL) was used for probe purification. A Cary Bio-300 UV spectrometer (Varian, Walnut Creek, CA) was used to measure absorbance for probe quantitation. Fluorescence measurements were performed on a Fluorolog-Tau-3 spectrofluorometer (Jobin Yvon, Inc., Edison, NJ). Cell images were conducted with a confocal microscope setup consisting of an Olympus IX-81 inverted microscope with an Olympus FluoView 500 confocal scanning system. A 40 × 0.6 NA air objective was used.

Molecular Probes Synthesis. All oligonucleotides were synthesized based on solid-state phosphoramidite chemistry at a 1 μmol scale. Both molecular probes listed in Table 1 were synthesized with controlled-pore glass columns with a 3′-Black Hole Quencher 2 molecule (BHQ2) covalently linked to the CPG substrate. The complete MB sequences were then deprotected in concentrated ammonia hydroxide at room temperature overnight and further purified with reverse phase high-pressure liquid chromatography (HPLC) on a C18 column with a linear elution gradient with TEAA (triethylammonium acetate) in acetonitrile changing from 20 to 70% over a 30 min period. The collection from the first HPLC separation was then

TABLE 1. Probes and Oligonucleotides Used in This Work[a]

name	sequence
MnSOD probe	5′-TTTTTTTTTTTTTTTTTTTT$(CH_2CH_2O)_{36}$Cy3-CGAGCCAGTTACATTCTCCCAGTTGATTGCTCGG−BHQ2−3′
random DNA probe	5′-Cy3-CCTAGCTCTAAATCACTATGGTCGCGCTAGG-BHQ2−3′
MnSOD cDNA	5′-AATCAACTGGGAGAATGTAACTG−3′
GT	5′-GTGTGTGTGTGTGTGTGTGTGTGTGTGT-3′

[a]The bases underlined are complementary to form the stems of the probes.

vaccuum-dried, incubated in 200 μL 80% acetic acid for 15 min, incubated with 200 μL of ethanol, and vaccuum-dried before the second round of HPLC. The HPLC was performed on a ProStar HPLC Station (Varian, CA) equipped with a fluorescent and a photodiode array detector.

Synthesis of GT/SWNT. Because of radioactive safety concerns and laboratory facility limitations, the SWNTs were cut before complexing with radioisotope-labeled GT sequence. The SWNTs were treated with strong acid mixture (nitric acid/sulfic acid = 3:1) and sonicated for 24 h. After washing with water, 1 mg/L treated SWNT was mixed with 0.1 μM GT sequence labeled with ^{32}P at room temperature and rocked for 24 h. The resulting complex was directly used for the digestion test.

Synthesis of MnSOD Probe/SWNTs. Since neutral SWNTs are the popular form for cellular application, we wanted to investigate its protection ability during cellular delivery. The SWNTs were ultrasonicated by sonic dismembrator (Fisher Scientific, Model 100) for 1 h. Then about 200 mg/L SWNT was mixed with 20 μM MnSOD probe aqueous solution. The mixture was sonicated for another 45 min to 1 h. Then the probe/nanotube solution was centrifuged at 22 000*g* for 6 h. The pellet comprising impurities and aggregates of nanotubes at the bottom of the centrifuge tube was discarded, and the supernatant was collected and ultracentrifuged for another 6 h at 22 000*g*. The supernatant is the MnSOD probe/SWNTs, which turned out to be soluble. After dialysis, the complex was stored at 4 °C. The solubilized SWNTs are not well-dispersed individual nanotubes, but the mixture of nanotube bundles and individual nanotubes.

Cellular Experimental Procedures. MDA-MB-231 cells (ATCC, HTB-26) were cultured in Leibovitz's L-15 medium with 2 mM L-glutamine supplemented with 10% fetal bovine serum (all reagents from Invitrogen). Cells were plated into chambered coverslides 1 day before the experiments so that cells would be about 90% confluence during the experiments. The concentrated MnSOD probe/SWNTs were added to each well at a final concentration of about 25 mg/L. The incubations were carried out at 37 °C air atmosphere for 12 h. After incubation, the cell medium was decanted from the well, and the cells were washed thoroughly. To stimulate MnSOD mRNA expression, cells were incubated in 1 μg/mL LPS from *E. coli* serotype 055:B5 (Sigma) for 4 h prior to cellular imaging. The control experiments were conducted under the same conditions, but without LPS stimulation.

Acknowledgment. This work was supported by National Institutes of Health grants, an ONR grant, and the State of Florida Center of Excellence grant.

REFERENCES AND NOTES

1. Sokolova, V.; Epple, M. Inorganic Nanoparticles as Carriers of Nucleic Acids into Cells. *Angew. Chem., Int. Ed.* **2008**, *47*, 1382–1395.
2. He, X. X.; Wang, K. M.; Tan, W. H.; Liu, B.; Lin, X.; He, C. M.; Li, D.; Huang, S. S.; Li, J. Bioconjugated Nanoparticles for DNA Protection from Cleavage. *J. Am. Chem. Soc.* **2003**, *125*, 7168–7169.
3. Martin, C. R.; Kohli, P. The Emerging Field of Nanotube Biotechnology. *Nat. Rev. Drug Discovery* **2003**, *2*, 29–37.
4. Cui, X. J.; Yu, S. H.; Li, L.; Li, K.; Yu, B. Fabrication of Ag_2SiO_3/SiO_2 Composite Nanotubes Using a One-Step Sacrificial Templating. *Adv. Mater.* **2004**, *16*, 1109–1112.
5. Pantarotto, D.; Briand, J. P.; Prato, M.; Bianco, A. Translocation of Bioactive Peptides Across Cell Membranes by Carbon Nanotubes. *Chem. Commun.* **2004**, 16–17.
6. Pantarotto, D.; Singh, R.; McCarthy, D.; Erhardt, M.; Briand, J. P.; Prato, M.; Kostarelos, K.; Bianco, A. Functionalized Carbon Nanotubes for Plasmid DNA Gene Delivery. *Angew. Chem., Int. Ed.* **2004**, *43*, 5242–5246.
7. Cai, D.; Mataraza, J. M.; Qin, Z. H.; Huang, Z. P.; Huang, J. Y.; Chiles, T. C.; Carnahan, D.; Kempa, K.; Ren, Z. F. Highly Efficient Molecular Delivery into Mammalian Cells Using Carbon Nanotube Spearing. *Nat. Methods* **2005**, *2*, 449–454.
8. Chen, C. C.; Liu, Y. C.; Wu, C. H.; Yeh, C. C.; Su, M. T.; Wu, Y. C. Preparation of Fluorescent Silica Nanotubes and Their Application in Gene Delivery. *Adv. Mater.* **2005**, *17*, 404–407.
9. Kwon, N. H.; Beaux, M. F.; Ebert, C.; Wang, L.; Lassiter, B. E.; Park, Y. H.; Mciiroy, D. N.; Hovde, C. J.; Bohach, G. A. Nanowire-Based Delivery of *Escherichia coli* O157 Shiga Toxin 1 A Subunit into Human and Bovine Cells. *Nano Lett.* **2007**, *7*, 2718–2723.
10. Hampton, T. Nanowire DNA Dlievery. *JAMA* **2007**, *298*, 505.
11. Seferos, D. S.; Giljohann, D. A.; Hill, H. D.; Prigodich, A. E.; Mirkin, C. A. Nano-Flares: Probes for Transfection and mRNA Detection in Living Cells. *J. Am. Chem. Soc.* **2007**, *129*, 15477–15479.
12. Liu, Z.; Winters, M.; Holodniy, M.; Dai, H. J. siRNA Delivery into Human T Cells and Primary Cells with Carbon-Nanotube Transporters. *Angew. Chem., Int. Ed.* **2007**, *46*, 2023–2027.
13. Kam, N. W. S.; O'Connell, M.; Wisdom, J. A.; Dai, H. J. Carbon Nanotubes as Multifunctional Biological Transporters and Near-Infrared Agents for Selective Cancer Cell Destruction. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 11600–11605.
14. Heller, D. A.; Jeng, E. S.; Yeung, T. K.; Martinez, B. M.; Moll, A. E.; Gastala, J. B.; Strano, M. S. Optical Detection of DNA Conformational Polymorphism on Single-Walled Carbon Nanotubes. *Science* **2006**, *311*, 508–511.
15. Medley, C. D.; Drake, T. J.; Tomasini, J. M.; Rogers, R. J.; Tan, W. H. Simutaneous Monitoring of the Expression of Multiple Genes inside of Single Breast Carcinoma Cells. *Anal. Chem.* **2005**, *77*, 4713–4718.
16. Zheng, M.; Jagota, A.; Semke, E. D.; Diner, B. A.; Mclean, R. S.; Lustig, S. R.; Richardson, R. E.; Tassi, N. G. DNA-Assisted Dispersion and Separation of Carbon Nanotubes. *Nat. Mater.* **2003**, *2*, 338–342.
17. Yang, R.; Jin, J.; Chen, Y.; Shao, N.; Kang, H.; Xiao, Z.; Tang, Z.; Wu, Y.; Tan, W. Carbon Nanotube-Quenched Fluorescent Oligonucleotide: Probes that Fluoresce upon Hybridization. *J. Am. Chem. Soc.* **2008**, *130*, 8351–8358.
18. Nakayama-Ratchford, N.; Bangsaruntip, S.; Sun, X.; Welsher, K.; Dai, H. Noncovalent Functionalization of Carbon Nanotubes by Fluorescein-Polyethylene Glycol: Supramolecular Conjugates with pH-Dependent Absorbance and Fluorescence. *J. Am. Chem. Soc.* **2007**, *129*, 2448–2449.
19. Li, J. W. J.; Fang, X. H.; Schuster, S. M.; Tan, W. H. Molecular Beacons: A Novel Approach to Detect Protein−DNA Interactions. *Angew. Chem., Int. Ed.* **2000**, *39*, 1049–1052.
20. Wu, Y.; Yang, C. J.; Moroz, L. L.; Tan, W. Nucleic Acid Beacons for Long-Term Real-Time Intracellular Monitoring. *Anal. Chem.* **2008**, *80*, 3025–3028.
21. Uchiyama, H.; Hirano, K.; Kashiwasake-Jibu, M.; Taira, K. Detection of Undergraded Oligonucleotides *in vivo* by Fluorescence Resonance Energy Transfer Nuclease Activities in Living Sea Urchin Eggs. *J. Biol. Chem.* **1996**, *271*, 380–384.
22. Tyagi, S.; Alsmadi, O. Imaging Natuve Beta-Actin mRNA in Motile Fibroblasts. *Biophys. J.* **2004**, *87*, 4153–4162.