Controlled Precipitation of Solubilized Carbon Nanotubes by Delamination of DNA

Robert J. Chen and Yuegang Zhang*

Intel Corporation, 2200 Mission College Blvd., Santa Clara, California 95054

Received: September 6, 2005; In Final Form: November 8, 2005

Polyaromatic molecules, such as rhodamine 6G and methylene blue, were found capable of precipitating DNA-solubilized single-walled carbon nanotubes from solution through a competitive binding mechanism whereby DNA is displaced from the nanotube surface, allowing the nanotubes to rebundle. This delamination of DNA also occurred when complementary oligonucleotides were used to hybridize specifically to the DNA coating on the nanotubes. These findings were expanded to include techniques for controlled desolubilization and to provide additional elucidation into the interaction of SWNTs and noncovalent solubilizing agents.

1. Introduction

Single-walled carbon nanotubes (SWNTs)[1,2] comprise a new class of nanomaterials that demonstrate remarkable versatility and wide applicability, most notably in new field-effect transistor architectures[3,4] and in other nanoelectronic applications such as chemo- and biosensing.[5,6] To leverage the full potential of SWNTs, techniques have been developed to solubilize the bulk-grown material into solution through a variety of noncovalent functionalization routes, thus enabling standard chemical and biological methods to be utilized.[7,8] These schemes have proven very effective and mark a major step forward in realizing new applications of bulk-grown SWNTs. However, while the solubilization has enabled a host of new capabilities, the ability to precipitate, or desolubilize, these SWNTs may prove equally vital down the road, for example, in post-separation procedures or in preparation for self-assembly onto a substrate to form SWNT devices.[9−12] The means to controllably precipitate a defined number of SWNTs with specific properties may prove just as critical in this regard. This report details the discovery and application of such a technique: the controlled precipitation of DNA-solubilized SWNTs by delamination of the DNA coating, a process to restore solubilized SWNTs back to their pristine nonfunctionalized form. Furthermore, the desolubilization can be modulated to allow either complete or partial precipitation of all SWNTs from solution or just a fraction, the latter of which leaves an SWNT mixture in solution that may be altered in some aspects of electronic and diameter distribution.

In this study, oligonucleotides, or single-strand DNA (ssDNA), were chosen as the solubilizing agent because of their ability to noncovalently functionalize and solubilize bulk-grown SWNTs into aqueous solution.[13,14] In contrast to surfactant solubilization,[7,8] DNA-solubilized SWNTs do not exhibit the tendency to precipitate upon dilution with water or by addition of organic solvents. This is due to the strong physisorption of DNA onto the SWNTs via a wrapping mechanism, which results in a very stable coating. Several factors contribute to this capability. One is that DNA possesses a highly negatively charged backbone comprised of sugar−phosphate linkages; this backbone imparts a great degree of hydrophilicity to the inherently hydrophobic SWNTs. This renders them water-soluble and simultaneously prevents coated SWNTs from rebundling, or aggregating, in solution through electrostatic repulsion. Additionally, this backbone can be substituted with the four possible DNA bases, ATCG (adenine, thymine, cytosine, and guanine, respectively). These relatively small molecules possess extended π structures which facilitate DNA binding to SWNTs through attractive $\pi-\pi$ stacking interactions.[15]

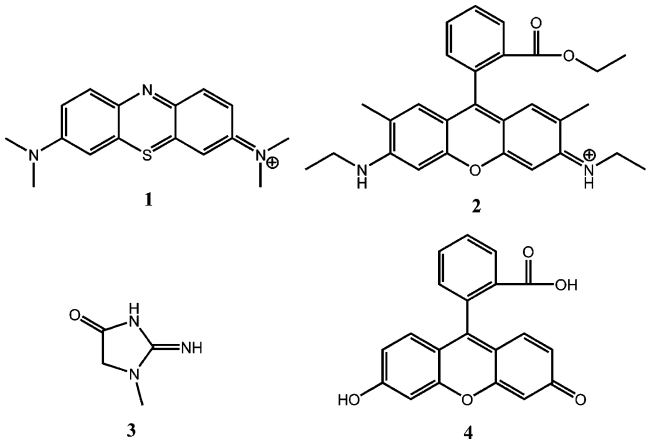

Figure 1. Methylene blue (MeB) (**1**) and rhodamine 6G (R6G) (**2**) are able to precipitate DNA-solubilized SWNTs from solution. Both molecules incorporate extended aromatic structures which competitively bind to the surface of SWNTs, thus actively desorbing DNA. Control experiments with creatinine (**3**) and fluorescein (**4**) verify that the extended aromatic structure is a requirement for the precipitation to occur and that the positive charge influences the kinetics of the process.

The first approach to the precipitation of DNA-solubilized SWNTs reported here explores the use of relatively small polyaromatic molecules. These molecules—methylene blue (MeB) (**1** in Figure 1) and rhodamine 6G (R6G) (**2** in Figure 1), among others—possess extended aromatic systems capable of forming attractive $\pi-\pi$ stacking interactions with SWNTs much in the same way as the DNA bases themselves. Thus, they are able to competitively desorb or displace the DNA coating from the SWNTs. The second approach builds on the insight gained from the small molecule approach and combines it with the advantage of DNA hybridization to actively remove DNA from the SWNTs.

* Author to whom correspondence should be addressed. E-mail: yuegang.zhang@intel.com.

10.1021/jp055044e CCC: $33.50
Published on Web 12/10/2005

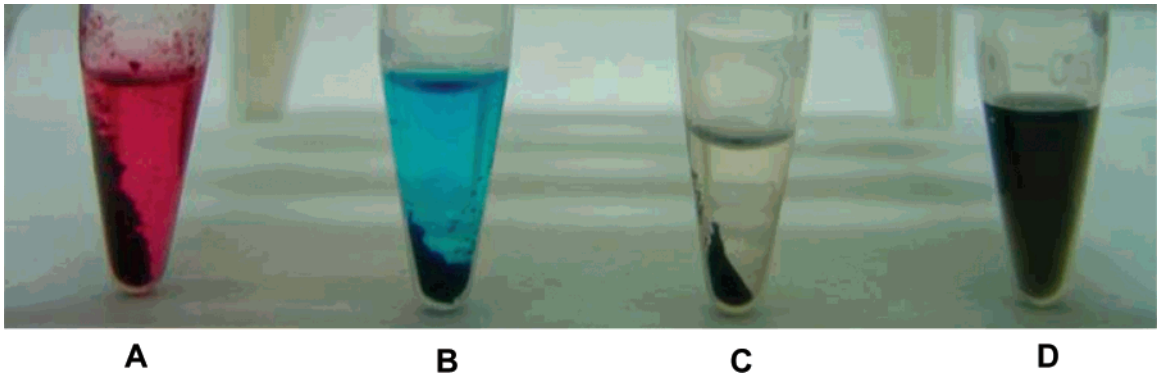

Figure 2. Photograph of a series of vials of centrifuged SWNTs after precipitation by (A) R6G, (B) MeB, and (C) PolyA30. (D) DNA-solubilized SWNTs after undergoing the same precipitation protocol as in A−C except for the absence of desolubilizing agent (R6G, MeB, or PolyA30).

2. Methods and Materials for SWNT Solution Preparation

Bulk HiPco SWNTs (Carbon Nanotechnolgies, Inc.) were first solubilized into aqueous solution with the aid of a custom-synthesized oligonucleotide (Operon Biotechnologies, Inc.) with sequence TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT (PolyT30). In a typical preparation, HiPco SWNTs (∼1 mg) were combined with a stock solution of PolyT30 (∼3 mL, 200 μM in 18 MΩ water) and were ultrasonicated in an ice water bath (VWR 75D bath sonicator) for 2−3 h at a power of 90 W with frequent vortexing interspersed throughout. The solution was then centrifuged at 16 000*g* for 90 min, and the supernatant was decanted and collected. This was repeated three times, following which the resulting supernatant was dialyzed (MWCO 60 000) against pure water for a period of 2−3 days to remove any free DNA not coated directly on the SWNTs. This procedure typically afforded a final concentration of 0.1−0.2 mg/mL of solubilized SWNTs, representing a solubilization yield of 5−10%. The prepared SWNT solution was used for controlled precipitation experiments which are described in the following sections.

3. Results and Discussion

3.1. Dye-Mediated Desolubilization. Precipitation of the solubilized SWNTs by R6G or MeB was carried out by adding small aliquots (2−50 μL, 1−10 mM in water) to a DNA-SWNT solution (∼0.5 mL) to afford a final concentration of 25−250 μM R6G or MeB. The mixture was agitated gently for 2−3 h followed by centrifugation at 12 000*g* for 10 min at 15 °C, after which the top half of the supernatant was then carefully decanted and collected for characterization. At high dye concentrations (∼250 μM), spontaneous precipitation began within minutes and complete aggregation occurred after a few hours, typically producing a clump of SWNTs floating at the surface. After centrifugation, this clump was compacted into an insoluble pellet leaving behind a completely transparent solution above (Figure 2A, 2B). At lower concentrations, the precipitation can be controlled to obtain a partial desolubilization of the DNA-SWNTs, the degree of which is roughly proportionate to the amount of R6G or MeB added. In this case, only a fraction of the DNA-solubilized SWNTs is removed from solution after centrifugation.

Those SWNTs that remained solubilized were then decanted and their radial breathing mode (RBM) signature was characterized by resonant Raman spectroscopy in the range of 200−400 cm^{-1} (Renishaw Invia triple wavelength spectrometer operating at 633 nm). This region of the spectrum is often used as a fingerprint for the electronic properties of SWNTs, from which their chiralities and diameters can then be ascertained.[16,17]

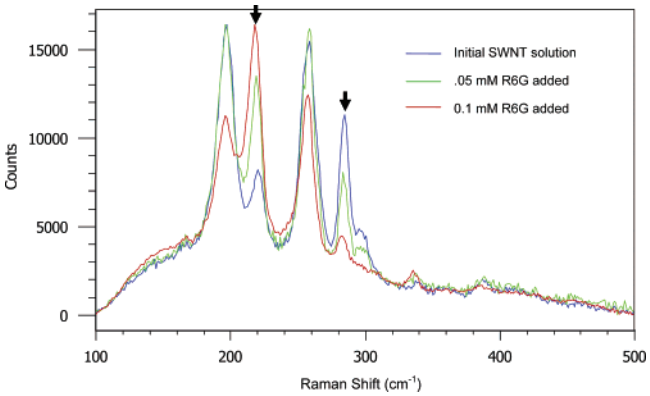

Figure 3. Spectra from Raman spectroscopy (at 633-nm wavelength excitation) showing the radial breathing mode (RBM) regime of SWNTs. Here, the evolution of certain peaks at 218.3 and 283.5 cm^{-1} (indicated by black arrows) is clearly seen to change dramatically with increasing concentrations of R6G.

SWNT solutions that had undergone partial desolubilization by R6G and MeB both exhibited dramatically altered RBM signatures. Figure 3 shows the case for R6G in which the change tracks the increasing concentration of R6G to which the solubilized SWNTs were exposed. Two peaks in particular (as indicated by the arrows) exhibit inverse trends with respect to increasing R6G concentration. These changes in the RBM signature may indicate a preferential desolubilization of SWNTs of particular chiralities or diameters. However, it may also be explained by a change of electronic structure of the SWNTs arising from the close proximity of R6G and the associated electron donation or withdrawal as well as field effects. Resonant Raman spectroscopy is particularly sensitive to such changes in the electronic distribution of the transition-energy levels.

Closer examination of the structures of both R6G and MeB reveals two major models for the mechanism underlying their ability to precipitate DNA-solubilized SWNTs. First is a simple electrostatic binding model in which R6G and MeB, both positively charged molecules by way of amino and imino moieties, are able to shield the negative charges on the DNA backbone and allow the SWNTs to approach each other, in essence acting as an electrostatic glue. This eventually leads to SWNT aggregation and precipitation. Another possibility is that R6G and MeB, with their extended aromatic structures, may well be able to compete with the DNA bases for SWNT $\pi-\pi$ surface interaction. This competitive binding mechanism necessarily implies a desorption of the DNA or delamination of the SWNTs through the interposition of R6G or MeB between the DNA strand and the SWNT surface. Rebundling and precipitation of the SWNTs would follow immediately because the small molecules by themselves cannot keep the SWNTs solubilized

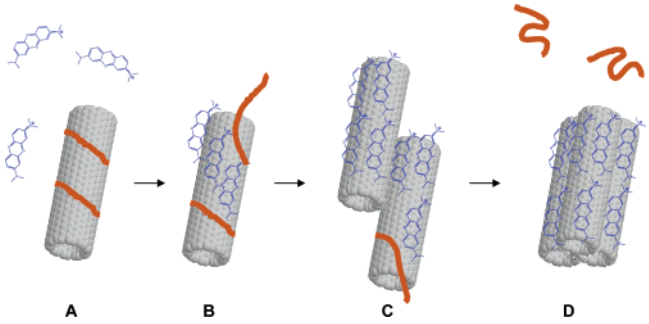

Figure 4. Schematic diagram illustrating the precipitation of DNA-solubilized SWNTs by methylene blue. (A) SWNTs are initially solubilized with PolyT30 ssDNA (in orange) coating. (B, C) MeB (in blue) competitively binds to the SWNT surface, displacing the DNA strands. (D) Delaminated SWNTs rebundle and precipitate out of solution as MeB is not an effective solubilizing agent.

in water (Figure 4). This is confirmed by control experiments wherein R6G and MeB alone were used in an attempt to solubilize SWNTs under the same protocol as DNA; no solubilization was observed.

To clarify the correct pathway then, precipitation studies were conducted with the molecules creatinine (**3**) and fluorescein (**4**). Creatinine was chosen because it possesses similar amino and imino moieties as found in R6G and MeB but lacks their extended aromatic structure, while fluorescein was chosen because it shares the extended aromatic structure of R6G and MeB but lacks their amino and imino moieties. In this way, structural and functional contributions could be isolated and probed as to their individual efficacies in the precipitation of DNA-solubilized SWNTs. The results were fairly conclusive. Creatinine effected no precipitation, even at extremely high concentrations (>100 mM), while fluorescein was successful albeit on a much slower time scale than R6G and MeB (days instead of hours). The insight gained here is twofold. The extended aromatic structure is a clear requirement for successful precipitation; however, a positive charge facilitates the process greatly, perhaps by operating to shield the negatively charged backbone of the DNA wrapping. Fluorescein, which is actually negatively charged, thus shows a much slower rate of precipitation as compared to its positively charged cousins R6G and MeB. Ultimately, both pathways contribute to the overall mechanism with favorable electrostatics allowing the competitive binding to occur much more quickly.

3.2. Hybridization-Mediated Desolubilization. If delamination is indeed the operating mechanism behind the ability of molecules such as R6G and MeB to precipitate out DNA-solubilized SWNTs, then any other method by which DNA can be removed from SWNTs should work also. One such alternative leverages the unique biochemistry and molecular recognition capabilities of DNA itself. Here, the underlying mechanism of desolubilization differs slightly from that of the small molecule case, however. In contrast to small molecule desolubilization, in which R6G or MeB insinuates itself between the DNA and SWNT surface and thereby competes off DNA, complementary ssDNA binds, or hybridizes, with the DNA coating itself, essentially peeling it off the SWNTs (Figure 5). The driving force for this action is the formation of the corresponding double-stranded DNA (dsDNA), a thermodynamically favored product because of the formation of hydrogen bonds between the bases of each ssDNA. This hydrogen bonding provides more than enough energetic motivation to overcome the comparably weaker $\pi-\pi$ stacking interactions between the bases and the SWNT surface.

In fact, complementary ssDNA does give similar precipitation results to R6G and MeB. Here, the complementary oligonucleotide PolyA30 was used, with the precipitation following a similar protocol for R6G and MeB, with the exception that typical final concentrations of PolyA30 were in the range of 2.5−25 μM to effect a similar degree of precipitation as seen with R6G and MeB. However, in this case, precipitation is seen to begin within hours instead of minutes. This is most likely due to the negative charge on the incoming ssDNA retarding the desolubilization process, much in the same way as seen previously with fluorescein. As with R6G and MeB, ssDNA-mediated desolubilization can also be modulated to allow for fractional precipitation of solubilized SWNTs. Figure 6 shows the vis-NIR spectra (Perkin-Elmer Lambda 900) of three equal aliquots of PolyT30-solubilized SWNTs after exposure to 2.5, 5.0, and 25 μM PolyA30. As can be seen, larger doses of ssDNA result in correspondingly larger drops in SWNT absorption as more and more are removed from solution. As a control, the completely noncomplementary oligonucleotide PolyC30 was also tried and had no effect on PolyT30-solubilized SWNTs. However, it was effective in analogous precipitation experiments with PolyG30-solubilized SWNTs as expected (data not shown).

Gel electrophoresis (Invitrogen E-Gel, 4% agarose) was used to confirm the presence of the dsDNA that would be expected to be produced in situ as a result of ssDNA-mediated desolubilization according to the mechanism shown in Figure 5. Figure 7 shows an image of the gel in which wells 7 and 8 were loaded

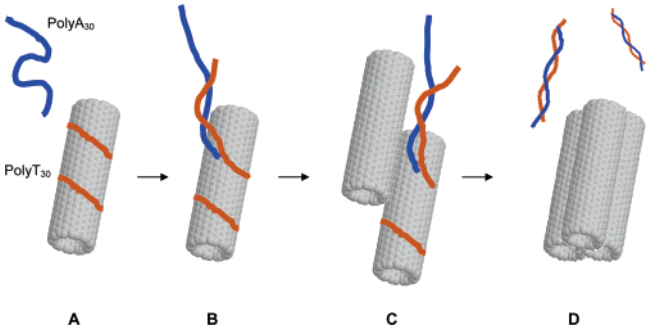

Figure 5. Schematic diagram illustrating the precipitation of DNA-solubilized SWNTs with complementary single-strand DNA. (A) SWNTs are initially solubilized with PolyT30 oligonucleotide (in orange) coating. (B) When the complementary oligonucleotide PolyA30 (in blue) is introduced, (C) desolubilization and precipitation of the SWNTs occurs, (D) leaving behind the corresponding double-strand DNA in solution.

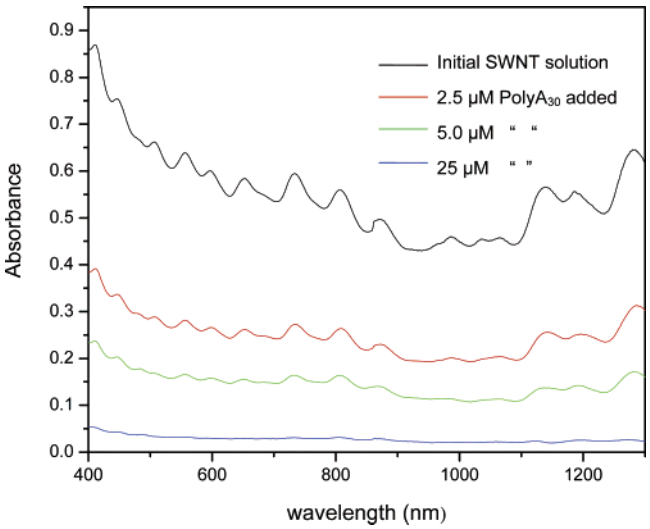

Figure 6. Vis−near-IR spectra showing incremental decreases in the concentration of PolyT30-solubilized SWNTs corresponding to increasing concentrations of PolyA30 (initial SWNT solution, black; 2.5 μM PolyA30, red; 5.0 μM, green; 25 μM blue).

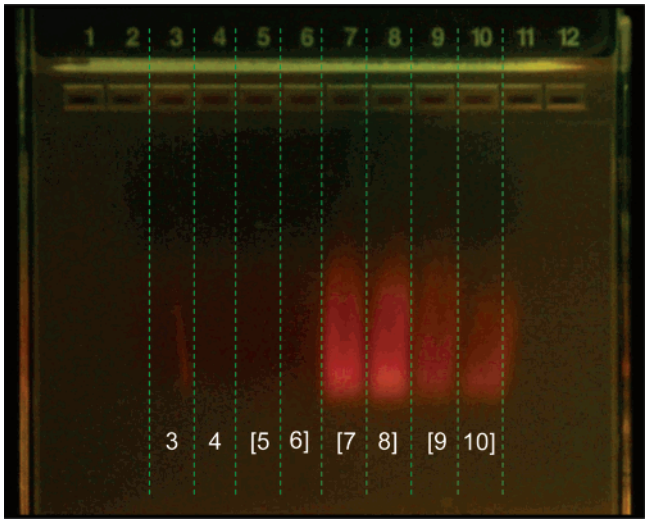

Figure 7. Photograph of an agarose gel (Invitrogen E-Gel, 4%) under UV illumination. Well 3 was loaded with the oligonucleotide PolyA30 (0.5 mM). Well 4 was loaded with the oligonucleotide PolyT30 (0.5 mM). Wells 5 and 6 were both loaded with an equal mix of two different oligonucleotides, PolyA30 + PolyC30 (total concentration 0.5 mM). Wells 7 and 8 were loaded with previously hybridized dsDNA (PolyA30/PolyT30) (0.5 mM). As is evident, the embedded dye in the agarose gel does not stain ssDNA as effectively as dsDNA at the same concentration leading to virtually no fluorescence signal in wells 3−6. However, dsDNA shows up strongly in wells 7 and 8. Wells 9 and 10 correspond to the supernatant of a solution of PolyT30-solubilized SWNTs that was fully precipitated with the complementary ssDNA PolyA30 (0.1 mM). The signals from these wells, while not as intense, show clear similarity to that of wells 7 and 8, thus indicating the presence of dsDNA.

with prehybridized dsDNA (PolyT30/PolyA30) as control samples and wells 9 and 10 were loaded with the supernatant from an SWNT desolubilization reaction. While the signals in wells 9 and 10 are less intense than that of wells 7 and 8 (because of concentration differences), the similarity is clear and confirms the presence of dsDNA, thus corroborating the mechanism outlined in Figure 5.

Hybridization-mediated precipitation departs from its dye-mediated counterpart in one significant aspect. Resonant Raman shows no significant change in the SWNT RBM region after partial precipitation by complementary ssDNA. (see Supporting Information); vis-NIR data also shows no change in adsorption peak ratios (vis-NIR spectroscopy cannot be applied to samples with dyes). This disparity can be rationalized as a consequence of the two different mechanisms proposed for the desolubilization: competitive binding versus delamination. Dye-mediated desolubilization operates under the competitive binding model (Figure 4), a process which is modulated by the degree of van der Waals interaction between the competing species and the SWNT. Thus, there may be sensitivity to factors that alter the nature of the SWNT surface such as its chirality and diameter. Delamination via hybridization, on the other hand, is determined primarily by the energetics of the DNA hybridization and not by preferential physisorption to the SWNT surface. As a result, hybridization-driven precipitation may not show the same selectivity as dye precipitation; however, there are still clear advantages in the former. Unlike with the small molecules R6G and MeB, there exists far greater potential for tailoring the degree of partial precipitation of SWNTs. Complementary oligonucleotides can be easily synthesized to incorporate base mismatches such that the strength of hybridization can be varied over a wide range from completely complementary to completely noncomplementary. This would afford unprecedented control over the desolubilization and, by extension, the final makeup of the remaining solubilized SWNTs.

4. Conclusion

This work serves to describe techniques aimed at the precipitation of solubilized SWNTs for integration into larger processes but more importantly provides significant insight into the interactions between noncovalent solubilizing agents, desolubilizaing agents, and SWNTs in aqueous solution. We recently have shown that the techniques described here can be generalized successfully to other types of SWNT solubilizers as well, such as glycosaminoglycan chondroitin sulfate and its derivatives.[18] Ultimately, investigations such as these into the nature of noncovalent SWNT surface interactions may prove key to the enabling of other technologies, such as the solution-phase separation and sorting of SWNTs and their self-assembly into integrated electronic devices.

Acknowledgment. We thank Colin Cai, Shida Tan, Herman Lopez, and Michael Stewart of the Intel Nanotube Strategic Research Project for their help in the preparation of DNA-solubilized SWNT samples and for useful discussion.

Supporting Information Available: Raman spectroscopy of PolyT30-SWNT solutions with addition of different concentration of PolyA30. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Iijima, S.; Ichihashi, T. *Nature* **1993**, *363*, 603.

(2) *Carbon Nanotubes: Synthesis, Structure, Properties, and Applications*; Dresselhaus, M. S., Dresselhaus, G., Avouris, P., Eds.; Springer: Berlin, 2001.

(3) Avouris, P. *Acc. Chem. Res.* **2002**, *35*, 1026.

(4) Appenzeller, J.; Knoch, J.; Derycke, V.; Martel, R.; Wind, S.; Avouris P. *Phys. Rev. Lett.* **2002**, *89*, 126801.

(5) Kong, J.; Franklin, N. R.; Zhou, C. W.; Chapline, M. G.; Peng, S.; Cho, K. J.; Dai, H. *Science* **2000**, *287*, 622.

(6) Chen, R. J.; Bangsaruntip, S.; Drouvalakis, K. A.; Wong Shi Kam, N.; Shim, M.; Li, Y. M.; Kim, W.; Utz, P. J.; Dai, H. *Proc. Natl. Acad. Sci.* **2003**, *100*, 4984.

(7) O'Connell, M. J.; Boul, P. J.; Ericson, L. M.; Huffman, C.; Wang, Y. H.; Haroz, E. H.; Kuper, C.; Tour, J.; Ausman, K. D.; Smalley, R. E. *Chem. Phys. Lett.* **2001**, *342*, 265.

(8) O'Connell, M. J.; Bachilo, S. M.; Huffman, C. B.; Moore, V. C.; Strano, M. S.; Haroz, E. H.; Rialon, K. L.; Boul, P. J.; Noon, W. H.; Kittrell, C.; Ma, J.; Hauge, R. H.; Weisman, R. B.; Smalley, R. E. *Science* **2002**, *297*, 593.

(9) Tan, S.; Lopez, H. A.; Cai, C. W.; Zhang, Y. *Nano Lett.* **2004**, *4*, 1415.

(10) Dyke, C. A.; Tour, J. M. *Nano Lett.* **2003**, *3*, 1215.

(11) Huang, L.; Cui, X.; Dukovic, G.; O'Brien, S. P. *Nanotechnology* **2004**, *15*, 1450.

(12) Strano, M. S.; Dyke, C. A.; Usrey, M. L.; Barone, P. W.; Allen, M. J.; Shan, H.; Kittrell, C.; Hauge, R. H.; Tour, J. M.; Smalley, R. E. *Science* **2003**, *301*, 1519.

(13) Zheng, M.; Jagota, A.; Semke, E. D.; Diner, B. A.; McLean, R. S.; Lustig, S. R.; Richardson, R. E.; Tassi, N. G. *Nat. Mater.* **2003**, *2*, 338.

(14) Zheng, M.; Jagota, A.; Strano, M. S.; Santos, A. P.; Barone, P.; Chou, S. G.; Diner, B. A.; Dresselhaus, M. S.; Mclean, R. S.; Onoa, G. B.; Samsonidze. G. G.; Semke, E. D.; Usrey, M.; Walls, D. J. *Science* **2003**, *302*, 1545.

(15) Chen, R. J.; Zhang, Y.; Wang, D.; Dai, H. *J. Am. Chem. Soc.* **2001**, *123*, 3838.

(16) Dresselhaus, M. S.; Dresselhaus, G.; Jorio, A.; Souza Filho, A. G.; Pimenta, M. A.; Saito, R. *Acc. Chem. Res.* **2002**, *35*, 1070.

(17) Bachilo, S. M.; Strano, M. S.; Kittrell, C.; Hauge, R. H.; Smalley, R. E.; Weisman, R. B. *Science* **2002**, *298*, 2361.

(18) Stewart, M. P.; Chen, R. J.; Zhang, Y. in preparation.