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Optical Absorption of DNA–Carbon Nanotube Structures

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ABSTRACT

We measured the UV optical absorption of single-stranded DNA bound to single-walled carbon nanotubes (DNA/SWNT). The nucleotide absorbance from DNA/SWNT provides the first experimental confirmation that DNA binds to nanotubes through π-stacking. Because the hypochromic absorbance typical of π-stacked structures are expected to occur primarily for DNA dipole transitions that lie along the axis of the optically anisotropic SWNTs, the absorbance changes following binding of DNA to nanotubes reveal the preferred orientation assumed by each of the four bound nucleotides with respect to the nanotube's long axis.

Zheng et al. reported that bundles of SWNTs in water can be effectively dispersed by sonication in the presence of single-stranded DNA (ssDNA).[1,2] Molecular modeling by that group suggested that DNA molecules hybridize with individual nanotubes by wrapping around them, with the interaction strength being provided by π-stacking, with the plane of the aromatic nucleotide bases oriented parallel to the surface of the nanotube.[1] The novel properties of this hybrid structure make it possible to separate a motley collection of nanotubes by electronic type,[1] an essential step toward nanotube electronic devices. The electrical properties of the hybrid may also be of special interest in the development of molecular-based electronic devices. Finally, nanotubes offer the potential of providing a scaffold on which DNA molecules can be oriented, manipulated, and studied without the need for chemical functionalization.

Although DNA/SWNT structures have been studied using optical absorption spectroscopy in the past,[1−7] little attention has been paid to the range of wavelengths of most interest for examining DNA, namely the UV region. DNA's UV absorbance has been studied extensively and has been central in understanding conformational changes of nucleic acids, such as the transition from double-stranded to single-stranded DNA.[8] Absorbance studies of DNA/SWNT samples have largely ignored the UV because, until not long ago, there was very little known about the absorbance properties of SWNT in this region. Some authors argue that the UV absorbance is entirely due to amorphous carbon material,[7] while others find the two absorbance peaks in the UV to be intrinsic to the nanotubes and, furthermore, to display a strong polarization dependence.[9] By the methods described here, we have found the UV absorbance peak locations and intensities to be repeatable under a variety of SWNT sample preparation conditions, and we demonstrate that the UV region provides important information about DNA/SWNT structure.

We used optical absorption spectroscopy from $\lambda = 190-900$ nm to explore the DNA−SWNT interaction of SWNT dispersed with DNA homopolymers of poly d(A_{30}), poly d(C_{30}), poly d(G_{30}), and poly d(T_{30}). Here, we report the first observation of anisotropic hypochromicity of optical transitions in DNA bases hybridized with SWNTs.

Thirty-base-long homopolymers were purchased from the Midland Certified Reagent Company (Midland, TX) as the ammonium salt in the form of a dry pellet and were hydrated with 50 mM phosphate buffer (P-buffer) solution, pH 7.5, to a concentration of 20 mg/mL. SWNTs (20 mg/mL, HiPCO, 0.8−1.2 nm diameter, Carbon Nanotechnologies Inc.) were added to these solutions, which were then ultrasonicated (Branson Cell Disruptor) surrounded by an ice bath for 30 min at a nominal power of 4 W. Centrifugation at 4 °C, 16 000 g, for 30 min was used to remove any pelleted, nondispersed carbonaceous material. During sonication, the amount of homopolymer bound to the SWNTs presumably reached saturation, as the addition of more DNA to the original solution did not increase dispersion of the SWNTs.

The supernatant was collected and applied to a buffer-equilibrated gel-filtration column (Sephacryl S-400 HR,

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exclusion limit of 271 bps) to remove any free, unbound DNA. We believe the resulting flow-through material consisted only of DNA/SWNT hybrids, as additional gel-filtration runs did not exclude any more homopolymers, as verified by UV−vis measurements and gel electrophoresis. The first gel-filtration step removed a considerable quantity of unhybridized homopolymer and was essential to ensure that the molecules being probed were those closely interacting with SWNT, not free, nonattached homopolymers. Because the absorptivity of the DNA/SWNT has not been established, we were unable to determine the DNA or SWNT concentration in this flow-through material, which was subsequently used on a volume basis.

To analyze DNA's contribution to the absorption spectrum of DNA/SWNT, the absorbance due to SWNTs had to be subtracted. We chose the absorption spectrum of sodium dodecyl sulfate-dispersed SWNTs (SDS/SWNT) to serve as our "bare SWNT" spectrum because SDS is known to disperse SWNT[11] without affecting the electrical properties of the nanotubes[12,13] and because the SDS molecule has featureless absorbance in the wavelength region of interest here. SDS/SWNT samples were produced by adding dry nanotubes to the P-buffer solution containing 1% by weight SDS and ultrasonicated for 30 min while surrounded by a room-temperature water bath. After sonication, the SDS/SWNT preparation was subject to centrifugation and gel-filtration, as had the DNA/SWNT preparations.

Optical absorption spectra were taken with a Shimadzu UV-260 recording spectrophotometer over a wavelength range of 190−900 nm and a cell path length of 1 cm. Samples (5 μL) of gel-filtered homopolymer/SWNT were added to 90 μL of DI H_2O. Reference cells contained 5 μL of the buffer solution in 90 μL DI H_2O to account for any absorption effects due to the buffer. Because of the inferior nanotube-dispersion efficiency of SDS, the absorption spectra of SDS/SWNT was determined directly in 90 μL of the undiluted gel-filtered preparation using a reference cell that also contained 90 μL 1% SDS in P-buffer. The absorbance spectra of each of the free homopolymers (30 μg/mL) was also determined.

Figure 1 shows the absorbance of all four DNA/SWNT samples from 200 to 800 nm. Absorption peaks arising from SWNT interband transitions occur in the visible region, while both the wrapped DNA and SWNT contribute in the UV region in the DNA/SWNT samples. From the strength of the absorption signal, it is clear that poly(dC) dispersed the SWNT the most efficiently. While we note that the dispersion efficiency can be affected by variations during the sonication procedure, subsequent sample preparations showed the same trend of dispersion efficiencies (poly(dC) > poly(dT) ∼ poly(dA) > poly(dG)). Synthesis of sequences containing a high percentage of guanine bases is notoriously difficult,[14] and it is possible that our poly(dG) solution contains fractionated species that are unable to disperse SWNTs. With each of the four homopolymers, we achieved unbundling and dispersion of the nanotubes, as evaluated by both AFM studies (that show SWCNT features like those previously published for DNA/SWNT samples[2]) and the absorption peaks in the visible due to SWNT interband transitions. For a sample of individually dispersed nanotubes, these absorption peaks are well resolved, whereas a sample consisting of bundles of nanotubes would exhibit a featureless absorbance or, at best, highly broadened peaks.[2,11] We note that the observed absorption properties of our samples are unaffected by extending sonication and centrifugation times up to 12 h for each.

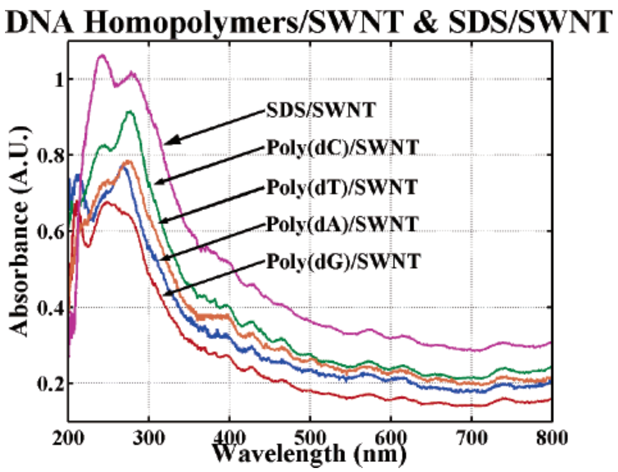

Figure 1. Absorbance spectra of SWNT dispersed by the four DNA homopolymers and SDS. Some absorbance spectra have been scaled for ease of comparison: poly(dA) (2×), poly(dT) (2×), poly(dG) (6×).

Figure 1 shows that the SDS also dispersed SWNTs, although with a much lower efficiency than the homopolymers do (recall that the volume of sample shown is 20 times that of the DNA/SWNT samples). Even with this lesser efficiency, the absorption peaks in the 350−800 nm region that are observed only in dispersed nanotubes[2,11] are evident, albeit less prominently than in the DNA/SWNT samples. The UV absorbance spectrum of our SDS/SWNT sample is consistent with Murakami et al.[9] and is highly repeatable, even under more vigorous sonication and centrifugation preparations. In addition, because we include a gel-filtration step in our preparation, any contaminants such as amorphous carbon would have to be both small enough to remain in solution during centrifugation yet larger than 8000 kDa to avoid being retained in the column. This leads us to conclude that the optical absorbance in this region is due to the nanotubes.

To establish the contribution of the attached DNA homopolymers to the overall absorbance, the absorbance due to the "bare SWNT" was subtracted from the spectra of the DNA/SWNT by scaling the SDS/SWNT absorbance spectra (∼5 times for poly d(C_{30}) to ∼20 times for poly d(A_{30})) such that their absorbances at 320 nm were equal to the DNA/SWNT 320 nm absorbance. The resulting absorbance (Figure 2) is referred to as that from the "bound" homopolymers. Substantial differences are observed between the absorbance spectra of the bound homopolymers and their free counterparts. For example, while the shape of the absorbance spectrum for poly(dA) looks similar in both the bound and free cases, the longer wavelength absorbance peak appears to have red-shifted by roughly 10 nm. Poly(dC) shows striking differences between bound versus free, with absor-

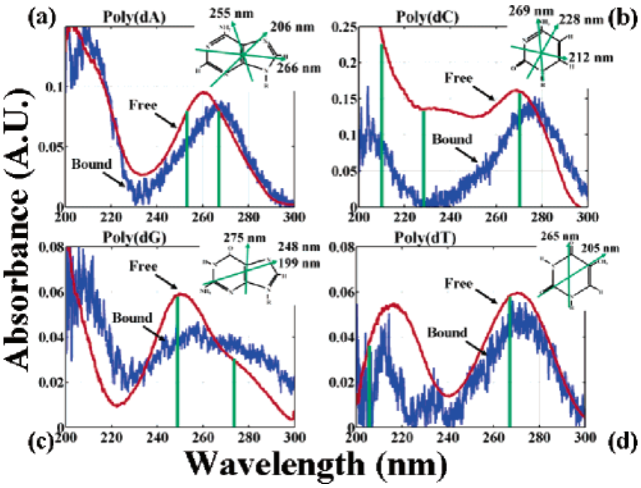

Figure 2. Absorbance spectra for each bound DNA homopolymer (blue) and each free one (red). Insets show the direction and wavelengths of optical dipole transitions. Wavelengths are indicated in plots (green).

bance in the region 220−240 nm largely disappearing when poly(dC) is hybridized with SWNTs. When hybridized with SWNTs, poly(dG)'s spectrum appears to have one broadened peak as opposed to the dominant peak with a shoulder it has when free. Poly(dT), conversely, appears mostly unchanged when hybridized with SWNTs. We explain the differences in these four samples as due to anisotropic hypochromicity of transitions in the DNA bases.

A feature common to all stacked chromophores is hypochromicity. Although complicated to describe quantitatively, a qualitative understanding of hypochromicity is straightforward. When one molecule absorbs light of a certain wavelength, a transitory dipole is formed in that species. This dipole in turn induces a dipole in the opposite direction of a neighboring stacked chromophore, which partly counteracts any dipole forming from the direct interaction with the incident light. Thus the stacked dimer structure has less absorbance than the sum of its two isolated components, leading to hypochromicity.[8]

Hypochromicity is often observed in dsDNA where DNA's UV absorption is due to in-plane $\pi-\pi^*$ transitions in the bases,[15] with contributions from the sugars and phosphates occurring mainly below 190 nm. Because these dipole transitions occur in all four of DNA's bases, all of these transitions are affected by hypochroism in the DNA, where the π-stacking between adjacent base pairs is the dominant energy term stabilizing the double helix.

The proposed π-stacking interactions between DNA bases and nanotube sidewalls modeled by Zheng et al.[1] is reminiscent of the aromatic stacking in dsDNA. But whereas in dsDNA each base stacks on top of another base, in DNA/SWNT structures, the bases are proposed to stack on the nanotube whose absorbance properties are very different than DNA. Murakami et al. have shown that there are two SWNT absorption peaks in the UV (centered at ∼275 and ∼236 nm) that are due to intrinsic optical properties of graphite and will thus be present in all SWNT samples.[9] Furthermore, they noted that these absorbance peaks exhibited a stark polarization dependence. SWNT are highly anisotropic, with a length-to-diameter ratio commonly up to 1000. As observed by several authors, this very great geometrical anisotropy leads to an absorptivity that depends very strongly on the optical electric field direction, with absorptivity along the tube axis calculated to be up to 20 times greater than absorptivity perpendicular to the tube axis.[10,16,17] Hence, for those DNA transitions whose induced dipolar moments align with the nanotube axis, we expect a strong hypochromicity as normally observed in stacked structures,[8] while for those transitions that align perpendicular to the nanotube axis, the hypochromicity will be suppressed. The insets of Figure 2 show the electric dipole transition moments for electronic transitions in the nucleic acid bases.[15] Because the sugars and phosphates of DNA's backbone do not absorb over the range of wavelengths examined here, these dipole moment values are representative of our ssDNA bound to SWNTs.

Now, re-examining the absorbance of poly(dG) (Figure 2c), the inset shows the directions and wavelengths of the electric transition dipole moments for guanine,[15] the base component that, along with a sugar and phosphate, repeats to form poly(dG). The dichroic ratio between the 248 and 275 nm peaks has changed once poly(dG) is hybridized with SWNT, with the absorbance at 248 nm being reduced more than at 275 nm. This indicates that, when the guanine bases stack on the nanotube sidewall, they do so with a tendency to align with the direction of the 248 nm transition moment along the nanotube axis.

Looking at the absorbance of poly(dA) (Figure 2a) in this manner, we see that what seemed at first as a red-shift may in fact be reduced absorptivity of one of the two transitions that contributes to adenine's 260 nm absorbance. As shown in the inset of Figure 2a, adenine's absorbance at 260 nm in free poly(dA) is actually the result of two transitions occurring at 255 and 266 nm. Hypochromicity of the 255 nm transition causes the absorbance peak of the bound poly(dA) to be centered on 266 nm, an apparent red-shift.

Poly(dC)'s absorbance curve is due to three major transitions in cytosine occurring at 212, 228, and 269 nm[15] (Figure 2b). While the transition at 269 nm appears to be unaffected by poly(dC)'s hybridization with SWNT, the 228 and 212 nm transitions are obviously attenuated when poly(dC) is bound to SWNT.

Poly(dT)'s absorbance spectrum remains mostly unchanged upon hybridizing with SWNT (Figure 2d), although there does appear to be a reduction of absorbance around 225 nm. This suggests the presence of a heretofore unknown transition at ∼225 nm. Not all of the DNA base transitions have been firmly established.[8]

This examination and analysis of the homopolymer spectra leads us to postulate that the DNA bases tend to orient themselves relative to the nanotube axis, as shown in Figure 3. We recognize that it is quite possible that DNA bases will have a distribution of orientations relative to the nanotube axis, if only due to the steric constraints of belonging to a polymer. But what is clear from our study is that binding of ssDNA to nanotubes results in marked

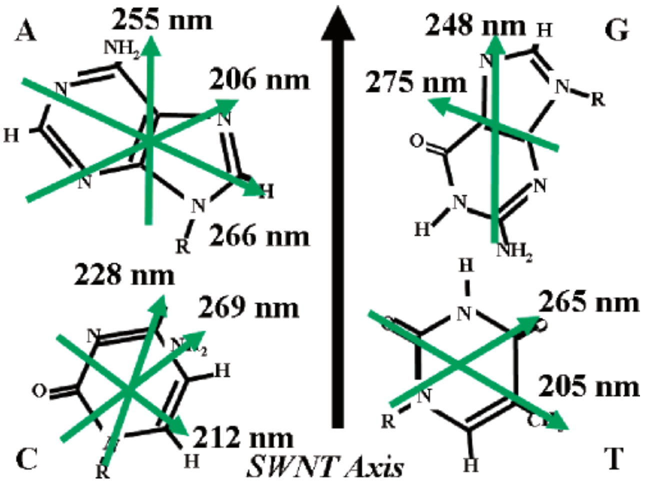

Figure 3. Orientation of the bound DNA bases relative to the SWNT axis, consistent with the data in Figure 2.

anisotropic hypochromicity that confirms the π-stacking of bases in DNA/SWNTs previously proposed[1] and reveals the preferred orientation assumed by each of the four bound nucleotides with respect to the nanotube's long axis. Computer calculations confirm that large hypochromicity effects are expected in DNA/SWNT hybrids.[18]

In summary, we have presented the optical absorbance spectra for SWNT dispersed by DNA homopolymers. We find that the anisotropic absorbance of SWNT leads to a large anisotropic hypochromicity in the stacked DNA bases and that the absorbance spectra of the bound DNA, and thus DNA base orientations, can be well explained using this description.

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References

(1) 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.

(2) Zheng, M.; Jagota, A.; Strano, M. S.; Santos, A. P.; Barone, P.; Chou, 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.

(3) Heller, D. A.; Jeng, E. S.; Yeung, T.-K.; Martinez, B. M.; Moll, A. E.; Gastala, J. B.; Strano, M. S. *Science* **2006**, *311*, 508.

(4) Gigliotti, B.; Sakizzie, B.; Bethune, D. S.; Shelby, R. M.; Cha, J. N. *Nano Lett.* **2006**, *6*, 159.

(5) Rajendra, J.; Baxendale, M.; Dit Rap, L. G.; Rodger, A. *J. Am. Chem. Soc.* **2004**, *126*, 11182.

(6) Rajendra, J.; Rodger, A. *Chem.*—*Eur. J.* **2005**, *11*, 4841.

(7) Huang, X.; Mclean, R. S.; Zheng, M. *Anal. Chem.* **2005**, *77*, 6225.

(8) Bloomfield, V. A.; Crothers, D.; Tinoco, I., Jr. *Nucleic Acids: Structure, Properties, and Functions*; University Science Books: Sausalito, CA, 2000.

(9) Murakami, Y.; Einarsson, E.; Edamura, T.; Maruyama, S. *Phys. Rev. Lett.* **2005**, *94*, 087402.

(10) Islam, M. F.; Milkie, D. E.; Kane, C. L.; Yodh, A. G.; Kikkawa, J. M. *Phys. Rev. Lett.* **2004**, *93*, 037404.

(11) 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.

(12) Islam, M. F.; Rojas, E.; Bergey, D. M.; Johnson, A. T.; Yodh, A. G. *Nano Lett.* **2003**, *3*, 269.

(13) Mickelson, E. T.; Chiang, I. W.; Zimmerman, J. L.; Boul, P. J.; Lozano, J.; Liu, J.; Smalley, R. E.; Hauge, R. H.; Margrave, J. L. *J. Phys. Chem. B* **1999**, *103*, 4318.

(14) Phillips, K.; Dauter, Z.; Murchie, A. I. H.; Lilley, D. M. J.; Luisi, B. *J. Mol. Biol.* **1997**, *273*, 171.

(15) Chou, P. J.; Johnson, W. C. *J. Am. Chem. Soc.* **1993**, *115*, 1205.

(16) Duesberg, G. S.; Loa, I.; Burghard, M.; Syassen, K.; Roth, S. *Phys. Rev. Lett.* **2000**, *85*, 5436.

(17) Hagen, A.; Hertel, T. *Nano. Lett.* **2003**, *3*, 383.

(18) Kaxiras, E., private communication.

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