BIOPOLYMER-MEDIATED ANALYTE DETECTION VIA PHOTOLUMINESCENCE MODULATION OF SINGLE-WALLED CARBON NANOTUBES

BY

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DISSERTATION

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ABSTRACT

Single-walled carbon nanotubes (SWNT) have unique photo-physical properties which, through the work in this dissertation, are investigated and harnessed to produce optical sensors with unique capabilities. Early studies of the modulation of SWNT optical properties—both photoluminescence and resonance Raman scattering—demonstrate their tunable nature. Solution dispersed SWNT are sorted by length and the photoluminescence quantum yield is shown to increase nonlinearly with length, suggesting that SWNT ends quench the exciton. The change in Raman scattering cross section and resonant window is mapped as a function of SWNT aggregation, as well as sonochemical effects on photoluminescence. Nanotube photoluminescence and scattering are then detected, via imaging and spectrometry, from within live murine macrophage cells, and shown to be extremely resilient, demonstrating the potential of nanotube-based molecular probes and biosensors. The work culminates in several major findings in optical sensing. We show that a nanotube-ds(GT)$_{15}$ DNA complex can detect genotoxic analytes by solvatochromism, and measure this from within live cells and tissues in real-time. We find that such optical signals can be multiplexed, resulting in analyte fingerprinting, and a bioanalyte can be detected at the single-molecule level stochastic operation of such sensors. These concepts are employed to detect, identify, and measure bioanalytes, such as reactive oxygen species, as well as explosives, such as TNT and RDX, with single-molecule sensitivity.
For Lorri, Douglas, Karen, Dorothy, and Milton Heller, and all my teachers
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CHAPTER 1: INTRODUCTION

Carbon nanotubes constitute one of the most promising materials born of the nanotechnological revolution. As nanotubes exhibit a simple composition and possess novel, and remarkable, properties absent in the bulk, they have captured the imagination of many researchers in a wide variety of fields. Learning to synthesize, measure, separate, and handle nanotubes has required extensive investigation, slowing the advent of much-anticipated commercial applications, however.

In 2002, the Smalley Group at Rice University discovered that single-walled carbon nanotubes emit near-infrared bandgap photoluminescence, a rare property among materials. Due to their 1-D electronic structure, the emission is environmentally sensitive as virtually all electron density involved in the photoluminescence lies at the tube surface. These characteristics give nanotubes potential for development into optical sensors. This dissertation explores the nanotube’s properties in an effort to develop a new class of sensors for analyte detection at unprecedented sensitivity and under unique conditions.

The thesis is divided as follows. Chapter 2 provides general background information on carbon nanotubes’ properties. Subsequent chapters provide background and literature pertinent to that chapter. Chapter 3 details work in the separation of nanotube length and diameter, sonochemistry and aggregation phenomena, and their effects on optical properties. Chapter 4 explores the potential for imaging and spectroscopy on nanotubes within live cells. Chapter 5 illustrates a novel sensor which exhibits the first example of solvatochromic shifting of nanotube photoluminescence as a detection mechanism. The sensor detects metal cations via responding to conformational changes of DNA on the nanotube surface. This sensor is also shown to function from
within live cells and tissue. Chapter 6 then demonstrates how nanotube-based sensors can detect and identify genotoxins and be employed in multiplexing—transmission of several signals simultaneously—from a mixture of nanotubes to identify analytes. It also details the first nanotube-based single-molecule stochastic sensor for the detection of a bioanalyte. In Chapter 7, we employ the previous concepts towards sensing and identification of several types of explosive and pesticide compounds containing nitro groups. The work details a new peptide-mediated detection mechanism and demonstrates a new microscope built to image solvatochromic shifts for ultra-sensitive detection.

The work exhibited herein is completed with the hope that nanotube-based optical sensors may prove to be useful tools in several research fields, and perhaps as instruments for medical diagnostics, forensics, defense, and security, to benefit the general public.
CHAPTER 2: BACKGROUND

2.1 SINGLE-WALLED CARBON NANOTUBE SYNTHESIS AND GEOMETRY

Single-walled carbon nanotubes (SWNT) are carbon nanostructures that consist of a single rolled sheet of graphene with diameters in the nanometer range. They were discovered in 1993 shortly after multi-walled carbon nanotubes.\textsuperscript{1,2} They have been synthesized via several methods including arc discharge, laser ablation of a graphite target, and chemical vapor deposition (CVD). The CVD method is most commonly used at present and the only one able to produce kilogram quantities of nanotubes per day.\textsuperscript{3} The high-pressure carbon monoxide (HiPco) method is the first to do so. It entails forming a metal catalyst in situ upon injecting Fe(CO)\textsubscript{5} or Ni(CO)\textsubscript{4} into a reactor kept at 900 to 1100 °C in a stream of carbon monoxide (CO) at 30 to 50 atm.\textsuperscript{4} This method produces SWNT with purities up to 97% in the diameter range of 0.7-1.1 nm. A method using a cobalt and molybdenum-based catalyst (CoMoCAT) produces a smaller diameter distribution where two nanotube species comprise 50% of the total SWNT produced.\textsuperscript{5} Nanotube production via CVD is under constant refinement. In the past few years, it has become routine to produce nanotube “forests” with lengths in the millimeters.\textsuperscript{6}

Each nanotube species be classified by two integers $(n,m)$ which define its diameter and electronic structure.\textsuperscript{7} By conceptually rolling and connecting point (0,0) with point $(n,m)$ on a graphene coordinate plane, all possible nanotube diameters and geometries can be formed (Figure 2.1).\textsuperscript{8} The chiral vector, $\mathbf{C}_h$, defines the nanotube species and can be expressed by real-space unit vectors $\mathbf{a}_1$ and $\mathbf{a}_2$ of the graphene lattice:

$$\mathbf{C}_h = n\mathbf{a}_1 + m\mathbf{a}_2 \quad (\text{where } n,m \text{ are integers and } 0 \leq m \leq n)$$ (2.1)
The chiral angle \( \theta \) of a nanotube is defined as the angle between \( C_h \) and \( a_1 \) and denotes the angle at which the hexagons are tilted along the nanotube axis. The chiral angle is described by:

\[
\cos \theta = \frac{C_h \cdot a_1}{|C_h||a_1|} = \frac{2n + m}{\sqrt{2n^2 + m^2 + nm}}
\]

(2.2)

When \( \theta = 0^\circ \) the nanotube is referred to as zigzag and when \( \theta = 30^\circ \), the nanotube is referred to as armchair, due to the patterns formed when looking perpendicular to the nanotube’s axis.\(^8\) Nanotube diameter relates to the \((n,m)\) indices according to:

\[
d = \frac{r_{C-C} \sqrt{3n^2 + nm + m^2}}{\pi}
\]

(2.3)

Where \( r_{C-C} = 0.144 \text{ nm} \), the carbon-carbon bond distance.

![Figure 2.1: The lattice structure of graphene. Each nanotube species is defined by joining point (0,0) with a point \((n,m)\) in the plane. The chiral vector, \( C_h \) joins these two points. The angle between unit vector \( a_1 \) and \( C_h \) is called the chiral angle of the nanotube.](image)
2.2 ELECTRONIC AND OPTICAL PROPERTIES

Graphene is a zero bandgap semiconductor whose Brillouin zone (reciprocal lattice) is shown in Figure 2.2. The high-symmetry points Γ, K, and M are shown. The valance and conducting bands of graphene touch at the K and K’ points. The nanotube electronic structure is obtained by the zone folding approximation, in which the wave vector along the circumference of the nanotube \( k_\perp \) is quantized while it remains continuous in the direction of the nanotube axis, \( k_z \). The allowed states are thus parallel sections of the graphene Brillouin zone. These lines depend on the \((n,m)\) indices of the nanotube, dictating the electronic character of the nanotube. If one of the allowed nanotube states intersects with a K or K’ point, where the valence and conducting band touch, the character of the nanotube will be metallic, but if they do not intersect, the nanotube will be semiconducting. In general, if \((n - m) \mod 3\) is 0, the nanotube will be metallic, while if it is 1 or 2, the nanotube will have a semiconducting electronic character.
Figure 2.2: The Brillouin zone of graphene showing high symmetry points. The zone folding approximation, which determines the structure of a carbon nanotube’s Brillouin zone, leads to quantized states along the circumference of the nanotube ($k_z$ wave vector). In the above case, the nanotube’s electronic structure is metallic as the cutting lines intersect the K point.\textsuperscript{11}

Due to the nanotube’s 1-D electronic structure, diameter-dependent van Hove maxima appear in the density of states (DOS).\textsuperscript{12,13} These spikes in the DOS are not present in the bulk (3-D) material, while a 2-D material gives a stepped shape in the DOS and a 0-D material exhibits discrete states. The electronic band structure of nanotubes is calculated by the tight-binding model, which is analogous to the linear-combination of atomic orbitals (LCAO) approach used in molecular physics.\textsuperscript{14} Semiconducting nanotubes possess an energy gap at the Fermi level ($E_F$) while metallic species do not. The DOS diagrams of both are illustrated in Figure 2.3.
Figure 2.3: Example density of states diagrams for a metallic nanotube (left) and a semiconducting nanotube (right). Semiconducting nanotubes contain an energy gap at the Fermi level ($E_f$). Electronic transitions ($E_{11}$, $E_{22}$) are allowed between bands of the same index (ie. $v_1 \rightarrow c_1$).

Absorption spectra of SWNT display sharp interband transitions associated with van Hove singularities. Allowed transitions occur from van Hove singularities of the same index, ie. between the first valence band (denoted $v_1$) and the first conduction band singularity ($c_1$). Together, this transition is denoted $E_{11}$. Experimentally, this transition is found to occur in semiconducting nanotubes at near-infrared wavelengths roughly between 800 – 1600 nm. The $E_{22}$ transitions occur at visible wavelengths, and $E_{33}$ occurs at ultraviolet wavelengths. Metallic $E_{11}$ transitions occur at ultraviolet and visible wavelengths. An absorption spectrum of a mixture of nanotubes produced by the HiPco process is shown (Figure 2.4). The convoluted nature of the spectrum stems from the large number of different nanotube ($n,m$) species in the heterogeneous sample. Large inter-nanotube van der Waals attractions often hinder individualization of nanotubes,
usually conducted by sonication in the presence of a surfactant-water solution. Nanotube aggregates elicit broadened absorption bands.

![Absorption spectrum of a suspension of carbon nanotubes synthesized by the HiPco method, suspended in surfactant.](image)

**Figure 2.4:** Absorption spectrum of a suspension of carbon nanotubes synthesized by the HiPco method, suspended in surfactant.

Semiconducting nanotubes display near-infrared fluorescence features in the 800 - 1600 nm range consistent with first van Hove (E\(_{11}\)) transitions.\(^8,15\) The emission is excitonic in nature.\(^16\) An electron-hole pair, or exciton, forms upon photoexcitation and emits a photon upon re-combining. The exciton forms upon excitation near the absorption maxima of the particular \((n,m)\) species. Although several absorption bands exist for each semiconducting nanotube, the exciton decays to the band edge, meaning all photoluminescence is governed by the \(E_{11}\) transition, relegating all emission to the near-infrared region. Photoluminescence linewidths are narrow (approx. 9 meV) when
measured for a single nanotube suspended in air or vacuum. In solution, however, the linewidth is closer to 25 meV due to inhomogenous broadening caused by environmental effects. The emission displays a small stokes shift from its absorption maximum, approximately 5 meV for most nanotubes. A common method for elucidating the nanotube photoluminescence maxima at different excitation wavelengths is to create a 3-D excitation-emission profile (Figure 2.5). Excitation maxima denote $E_{22}$ transitions, while emission maxima denote $E_{11}$ transitions. The assignment of nanotube $(n,m)$ indices to the photoluminescence maxima was achieved through a combinations of tight-binding model calculations and Raman spectroscopy.

**Figure 2.5**: A 3-D photoluminescence excitation-emission profile of surfactant-solubilized SWNT produced by the HiPco method.
The optical properties of SWNT have been seized upon by researchers for applications such as imaging\textsuperscript{19} and sensing\textsuperscript{20-22}. The first optical sensor was demonstrated by Barone, et. al. in which ferricyanide-doped nanotube responded to charge-transfer interactions upon reaction of the ferricyanide with glucose\textsuperscript{20}.  

2.3 LIST OF REFERENCES


CHAPTER 3:
TUNING SINGLE-WALLED CARBON NANOTUBE
STRUCTURAL AND OPTICAL PROPERTIES

3.1 INTRODUCTION AND BACKGROUND

Important advances in single-walled carbon nanotube (SWNT) separation\textsuperscript{1-9} are necessary for future development of nanotube-based technologies. Further advances in electronic,\textsuperscript{10-12} structural,\textsuperscript{13-15} and sensor\textsuperscript{16,17} applications will be dependent on the ability to exert precise control over SWNT diameter,\textsuperscript{6-8} length,\textsuperscript{1-5,9} and electronic structure.\textsuperscript{12,18,19}

3.1.1 Length and Diameter Separation

Significant advances in length\textsuperscript{1-5,9} and diameter\textsuperscript{6-8} separation of nanotubes have been made over the past few years. The most advanced separation work has been completed by Zheng and co-workers\textsuperscript{7,8} using a DNA wrapping procedure followed by ion exchange chromatography to yield nanotubes separated into distinct optical transitions. The basis of separation appears to be primarily by diameter, although some electronic component may also be operative.\textsuperscript{6} This technique may prove difficult to scale, however, because of the precise sequence of DNA needed for separation.

Length separation of nanotubes has been carried out using various chromatographic techniques.\textsuperscript{1-5,9} Size exclusion chromatography of raw SWNT material has produced fractions of individual and bundled nanotubes with mean lengths around 200 nm.\textsuperscript{4,5} Capillary electrophoresis (CE) produces length-separated fractions, the shortest consisting of nanotubes averaging less than 250 nm.\textsuperscript{1,2} In order to enhance length separation, SWNT cutting has been achieved using nitric acid to shorten nanotubes.
from the ends.\textsuperscript{5} This method also causes significant functionalization and damage of the nanotube sidewall, however, which perturbs the electronic structure.\textsuperscript{20}

3.1.2 Raman Spectroscopy of SWNT

The peaks in the low wavenumber region of the SWNT Raman spectrum are frequently used as a measure of the relative populations of particular carbon nanotubes\textsuperscript{21-23}. The known diameter dependence of these radial breathing modes (RBM) allows for almost a direct identification of particular (n,m) species in the sample that are resonant at the particular excitation wavelength\textsuperscript{24,25}. Several recent investigations have compared relative changes to the intensities of these modes in the effort to benchmark separation processes\textsuperscript{19,26,27}. Additionally, the tangential mode region between 1500 to 1600 cm\textsuperscript{-1} in the Raman spectrum can identify the presence of metallic and semiconducting nanotubes as the former gives rise to a Breit-Wigner-Fano (BWF) lineshape. Here, the discrete tangential phonon couples to the continuum of electronic states at the Fermi level\textsuperscript{21,23,28}.

Despite the widespread use of these features in benchmarking separation processes, several complications arise that may lead to inaccurate conclusions regarding separation. Moreover, understanding the relationship between aggregation and spectroscopy would benefit efforts to functionalize nanotubes\textsuperscript{19}, make well-dispersed nanotube composites\textsuperscript{29}, or use Raman spectroscopy to monitor other nanotube processing techniques. The strong resonant enhancement of the SWNT Raman spectrum is well documented\textsuperscript{21-24}. Samples in different physical\textsuperscript{25} or chemical\textsuperscript{30} environments can exhibit variations in the relative intensities of various Raman modes. Previous investigations
have explored the effects of aggregation\textsuperscript{31} and chemical environment\textsuperscript{30,32,33} on these spectra.

3.2 LENGTH SEPARATION

Herein, we demonstrate a scalable method for generating length and diameter-separated carbon nanotubes via ultrasonication and chromatography. Material is characterized by atomic force microscopy (AFM) and Raman, fluorescence, and absorption spectroscopies. The cutting process in our system is found to be diameter-selective, causing length fractions to exhibit enrichment or depletion of nanotubes of certain widths. Specifically, we find that shorter nanotubes are enriched in large-diameter species, while longer nanotube fractions are enriched with small-diameter tubes.

Gel Electrophoresis using field strengths of 7 V/cm for 30 minutes was performed on carbon nanotubes produced by the HiPco process, suspended in water with sodium cholate and sonicated for 5 different durations. The SWNT samples, initially ultrasonicated for 10 minutes, then subsequently probe-tip sonicated at for an additional 0 hrs, 1 hr, 3 hrs, 5 hrs, and 10 hrs, result in different degrees of nanotube migration into the gel (Figure 3.1). The resulting agarose gel, run in TAE buffer with 50 mM sodium cholate, shows that faster migration results as the probe-tip ultrasonication duration increases. In the control (0 hrs), material appears closest to the sample wells with some material failing to migrate out of the well at all. In the lane containing nanotubes probe-tip sonicated for 10 hours, material concentrates further from the wells and leaves no residual material in them. The other samples show a systematic increase in migration with ultrasonication time.
Figure 3.1: Agarose gel run for 30 minutes containing nanotubes probe-tip sonicated for various durations. The material in the lane labeled “0 hrs” is cup-horn sonicated for 10 minutes with no further probe-tip ultrasonication. The other lanes contain solutions similarly cup-horn sonicated and additionally probe-tip sonicated for 1, 3, 5, and 10 hours.

AFM measurements of nanotubes electroeluted from a gel run under identical conditions (7 V/cm, 30 min, 10 hr ultrasonication), reveal length-dependent separation (Figure 3.2). The first fraction of electroeluted material, which moves at the highest velocity from the sample wells, contains nanotubes with an average length of 92 nm. Nanotubes in fraction F2 average 144 nm, while those in F3 average 254 nm. The mean length appearing in fraction 6 is 435 nm, but the standard deviation of lengths increases greatly for the later fractions. We conclude from these measurements that gel electrophoresis separates nanotubes primarily by length, as the shortest nanotubes move most quickly through the gel, and that ultrasonication cuts nanotubes in proportion to the duration of ultrasonic processing.
Figure 3.2: Atomic force micrographs of 6 electroeluted gel fractions of nanotubes sonicated for 10 hours. Material is removed from a set of wells placed 4.5 cm from the original sample wells. F1 is the first eluted fraction. Each successive fraction elutes after five additional minutes of applied potential. Average nanotube lengths and standard deviations are listed for each fraction. All images use the same scale.

3.3 DIAMETER SEPARATION

The heights of 20 nanotubes on the silicon substrate were measured from each of fractions 1, 3, and 6 via AFM. The mean heights are 0.829 nm, 0.789 nm, and 0.585 nm with standard deviations of 0.134 nm, 0.201 nm, and 0.146 nm respectively. These values appear low compared with the range of typical HiPco diameters (0.6 to 1.2 nm) despite calibration using etched mica and controlling for deformation due to tip compression.
Burghard and co-workers suggest that ATPES, used to adhere SWNT to the silicon surface, can mask heights as nanotubes recess within the monolayer. Nevertheless, the trend of relative heights suggests that shorter nanotubes, which move more quickly in the gel, possess larger diameters (by 30%).

**Figure 3.3:** Diagram of apparatus used for Raman/fluorescence spectroscopy on agarose gels. The gel sits on a translation stage which moves 0.5 mm between spectra taken through a probe head in which excitation and emission light use the same aperture.

Raman and fluorescence data of nanotubes in the gels were measured for the 10-hour sonicated sample after voltage was applied for 1 hour. Using an automated translation stage, Raman/fluorescence spectra at 785 nm excitation were taken every 0.5 mm from the sample well to the end of the gel lane (Figure 3.3). Figure 3.4 presents the normalized intensities of four different Raman and one fluorescence peak plotted versus distance from the sample wells (top plot). The maximum of each feature occurs at different distances. The 213 cm$^{-1}$ (9,7) ($d = 1.103$ nm) feature reaches its maximum intensity at approximately 64.5 mm into the gel. The 231 cm$^{-1}$ (12,1) ($d = 0.995$) RBM reaches its maximum at 61.5 mm from the well. These preferential migrations occur only
for nanotubes exposed to extended probe-tip ultrasonication. The bottom of Figure 3.4 shows migration of material subjected to cup-horn ultrasonication for 10 minutes only.

Figure 3.4: Profiles of five Raman and fluorescence nanotube features and selected Raman RBM spectra in an agarose gel run for 1 hour with 100 V applied potential. Peak heights are recorded versus distance from the sample well. Profiles are normalized with respect to the maximum height of each feature. (Top) Profile of nanotubes probe-tip sonicated for 10 hours. (Bottom) Profile of nanotubes probe-tip sonicated for 0 hours.
In a gel containing material sonicated for 10 hours, a Raman spectrum at 71.5 mm from the sample wells shows the (9,7) and (12,1) features at approximately equal intensity (Figure 3.5c). The high relative intensity of the (9,7) RBM, normally diminutive in a HiPco sample, implies enrichment of the large (9,7) nanotube at this position in the gel. Conversely, a Raman spectrum at 45 mm from the origin shows only the smaller (12,1) RBM, implying this species’ slower average movement through the agarose gel (Figure 3.5a). The 223 cm\(^{-1}\) RBM, corresponding to the (10,5) nanotube, (d = 1.050 nm), reaches its maximum at the same location as the (12,1) feature, though in all samples of intermediate ultrasonication times (1-5 hours), the peak appears to migrate faster than the (12,1) RBM and more slowly than the (9,7), consistent with other observations of diameter-dependent Raman peak migration. Analogous RBM changes are found in Raman spectra taken at 633 nm excitation (Figure 3.6). The effect is apparently independent of the surfactant used. Nanotubes suspended and sonicated in sodium dodecyl sulfate (SDS) show similar relative changes in the RBMs to sodium cholate-suspended tubes if electrophoresed with sodium cholate or Triton X-100 surfactant as the running buffer. We note, however, that if SDS is used as a running buffer, nanotubes flocculate in the gel.
Figure 3.5: (a-c) Spectra of Raman RBM regions of nanotubes, probe-tip sonicated for 10 hours, taken (a) 45 mm, (b) 61 mm, and (c) 71.5 mm from the sample wells. (d-e) RBM spectra of nanotubes probe-tip sonicated for 0 hours taken (d) 46 mm, and (e) 73 mm from the sample wells. (An analogous spectrum to (a) was not found.)
Figure 3.6: Radial breathing modes of carbon nanotube fractions electroeluted from agarose gel. Excitation was at 633 nm.
In the agarose gels, Electrophoretic mobility, $\mu = \frac{q}{f}$, depends on the molecular charge, $q$, and on the frictional coefficient, $f$, which is dependent on the solution viscosity and the molecular size of the analyte. To elucidate the cause of the mobility increase, size exclusion chromatography was used, as this technique separates species solely by molecular size. A sample sonicated for 3 hours was run through a 100 cm size exclusion column (ID = 1.5 cm) using high resolution gel filtration media composed of allyl dextran and N,N’-methylene bisacrylamide. The eluate was fed by a peristaltic pump at 0.5 mL per minute through absorption and Raman spectrometers to record a UV-Vis-NIR spectrum every four minutes and a 785 nm Raman spectrum every 30 seconds (Figure 3.7). Raman and fluorescence profiles of peak intensity versus elution time in the column (Figure 3.8, top) show qualitatively similar behavior to electrophoresed nanotubes sonicated for 3 hours (Figure 3.8, bottom). All nanotube species elute in the same order using both gel electrophoresis and size exclusion chromatography and analogous RBM spectra are found in both techniques (Figure 3.9). We therefore conclude that the distinct electrophoretic mobilities of nanotubes result from molecular size differences only. Since length contributes to the vast majority of size differences in nanotubes, the mobility is deemed largely length-dependent.
Figure 3.7: Diagram of size exclusion chromatography experiment. A 100 cm gravity flow column elutes into a tube connected to a Raman spectrometer and UV-Vis-NIR spectrophotometer. A peristaltic pump draws the eluate through the apparatus and into a fraction collector.
Figure 3.8: (Top) Profile of five Raman and fluorescence peaks during a size exclusion chromatography experiment using nanotubes sonicated for 3 hours. (Bottom) Profile of an agarose gel containing nanotubes sonicated for 3 hours run at previous conditions. Profiles were normalized with respect to the maximum height of each feature.
Figure 3.9: Radial breathing modes at 785 nm excitation of nanotube fractions electroeluted from agarose gel. The sample was probe-tip sonicated for 10 hours prior to electrophoresis.

Absorption spectra of the size exclusion column-separated nanotubes show that the diameter distribution of nanotubes is changing across fractions (Figure 3.10). Focusing on the E$_{11}$ transition peaks (900 nm and higher), we note that transition wavelength is roughly monotonic with respect to diameter in this region. Using this assumption, we see that the fraction eluted at 60 minutes is enriched in small diameter nanotubes when compared to the starting material. However, during elution, the long wavelength peaks at 1280 nm and 1185 nm increase with a concomitant decrease in short
wavelength peaks at 980 nm, 1036 nm, and 1130 nm. This implies a change in diameter distribution with elution time. Absorption spectra of nanotubes electroeluted from agarose gels show behavior analogous to column-separated nanotubes (Figure 3.11). In the E_{11} region again, short wavelength peaks appear in early fractions, followed by longer wavelength peaks in later fractions. Absorption spectra therefore supplement the argument for diameter separation.

Figure 3.10: Absorption spectra of selected fractions of nanotubes, probe-tip sonicated for 3 hours, and separated by a size exclusion column. Spectra show changes in concentrations of nanotube species with respect to elution time.
Figure 3.11: Absorption spectra of the six nanotube fractions electroeluted from an agarose gel. Fraction F0 is the starting material.

Changes in nanotube optical spectra have been correlated to various phenomena other than selective enrichment or depletion of certain species. For example, bundling (aggregation) of nanotubes changes the Raman resonances due to electronic dispersion between tubes in contact with each other. By corroborating Raman changes with absorption and fluorescence spectroscopies, however, this possibility can be eliminated. Fluorescence spectra of electroeluted fractions taken from 900 to 1400 nm emission are compared to absorption and Raman spectra (Figure 3.12). At 650 nm excitation, fluorescence shows analogous changes to the absorption spectra. The large diameter nanotubes (9,7), (10,3) and (8,6) appear in the first electroeluted fraction, F1. It should be noted that the (9,7) and (10,6) features are off-resonance at 650 nm, though
they appear at equal or greater intensity than other peaks in the spectrum. The spectrum of F5 shows that the three large diameter peaks are absent or dwarfed by the smaller diameter nanotube features: (8,3), (7,5), and (7,6).

![Near-infrared fluorescence spectra of the six fractions electroeluted from an agarose gel and the starting material, taken at 650 nm excitation.](image)

**Figure 3.12:** Near-infrared fluorescence spectra of the six fractions electroeluted from an agarose gel and the starting material, taken at 650 nm excitation.
Raman, absorption, and fluorescence spectra of gel and column fractions all suggest that nanotube species of larger diameters move in unison with small molecular sizes. Separation by diameter is thus concomitant with length fractionation, and nanotubes that have been cut shortest also possess the greatest relative enrichments of large diameter species. As longer ultrasonication time causes increased electrophoretic mobilities in the gels, we credit ultrasonication for determining the degree of both length and diameter separation of the nanotubes.

Our diameter-selective cutting results run counterintuitive to certain chemical and mechanical arguments. Ultrasonication is believed to cut nanotubes because collapsing cavitation bubbles create localized areas of high pressure and temperature in the vicinity of the nanotube sidewall. Continuum mechanics arguments are used to model this cutting process. Shear and normal forces are calculated on a hypothetical hollow cylinder. Normal stresses are determined by

$$\sigma = \frac{M}{\pi r^2 t}$$  \hspace{1cm} (3.1)

where $M$ is the bending moment on a cylinder, $t$ is the thickness of the wall (held constant), and $r$ is the average of outer and inner radii. Shear stresses are modeled using

$$\tau_{\text{max}} = \frac{4V}{3A} \frac{3r^2 + 3rt + t^2}{4r^3 t + 6r^2 t^2 + 4rt^3 + t^4}$$  \hspace{1cm} (3.2)

where $V$ is the shear force acting on a cross section and $A$ is the cross sectional area: $\pi(r_2^2 - r_1^2)$.

Relative shear and normal forces calculated for the nanotubes measured by Raman spectroscopy predict the opposite cutting behavior of that observed spectroscopically, as the calculated stresses decrease with increasing nanotube diameter.
Also in disagreement with our separation results are the pyramidalization and $\pi$-orbital misalignment angles.\textsuperscript{39} Both are inversely proportional to nanotube diameters and directly proportional to predicted reactivities of nanotube species. Smaller diameters, according to these indicators, should lead to decreasing stability and shorter nanotubes. Therefore, strain caused by pyramidalization or $\pi$-orbital misalignment cannot explain SWNT cutting by ultrasonication.

Zhang and Iijima report that the larger diameter SWNT prepared by laser ablation contain more defects than smaller diameters.\textsuperscript{40} Similar behavior during the HiPco process could explain preferential cutting of nanotubes during ultrasonication, as nanotubes weakened by defects may be cut more easily. Alternatively, Miyauchi et. al. illustrates the unusual stability imparted to some small-diameter nanotubes by endcaps which follow the isolated pentagon rule, used to describe stable fullerene structures.\textsuperscript{41} Experiments designed to elucidate the mechanism of diameter-dependent cutting are in progress.

We assert that selective depletion and enrichment of certain nanotube species has occurred to our fractions, though changes in optical properties of carbon nanotubes with length fractionation could arguably be attributed to various length-dependent optical effects.\textsuperscript{42} For instance, Rochefort, et. al. predicts an increase in metallic nanotube bandgap with decreasing nanotube length using ab initio and semiempirical calculations on zig zag $(n=m)$ nanotubes. According to Rochefort, finite-length effects do not change the bandgap until nanotubes are 5-10 nm in length, corresponding to the 1-D to 0-D transition. Scanning tunneling microscopy experiments show that bandgap changes
occur when nanotubes are cut to a few tens of nanometers.\textsuperscript{43} Therefore, it is unlikely that we are seeing these particular finite-length effects in our nanotubes.

3.4 RELATIVE QUANTUM YIELD OF SEPARATED NANOTUBES

All fluorescence features measured in the agarose gel migrate approximately 44 – 45 mm into the gel, though RBM maxima elute approximately 20 mm farther down the lane. Five fluorescence features are measured (corresponding to the (8,3), (6,5), (7,5), (6,4) and (9,1) nanotubes), and all curves exhibit similar shapes. For clarity, only the (8,3) peak is shown as all fluorescence features exhibit approximately the same behavior as the (8,3) peak. Fluorescence features measured by this method correspond to smaller diameter nanotubes than those appearing in the Raman spectra, potentially explaining the early maxima relative to the RBM modes (i.e. these small diameter nanotubes are disproportionately longer.). However, the early maxima and subsequent intensity decrease with the tube lengths in the gels and the column could also signify a decrease in quantum yield with the shortening of nanotubes.

A rough method of calculating relative quantum yields of nanotubes of different lengths was employed for the six fractions electroeluted from an agarose gel. Relative quantum yield is estimated using $\Phi_r = \frac{F_{\text{feature}}}{A_{\text{feature}}}$ where $A_{\text{feature}}$ and $F_{\text{feature}}$ are the absorbance and fluorescence intensity of the sample at the center wavelength of a particular nanotube transition. Due to the convoluted nature of the absorption spectrum, this is inexact. The relative quantum yields of the fractions are calculated using an absorption peak at 1036 nm and a fluorescence peak at 1028.50 nm, corresponding to the (7,5) nanotube, as well as an absorption feature at 1125 nm and fluorescence at 1122.88
nm, consistent with the (7,6) nanotube. In both cases, quantum yield is found to increase exponentially with respect to average nanotube length (measured via AFM) (Figure 3.13).

Figure 3.13: Relative quantum yields of the (7,5) and (7,6) nanotubes, electroeluted from an agarose gel, plotted versus average nanotube length as measured by AFM.

3.5 ULTRASONICATION-INDUCED CHANGES IN SWNT PHOTOLUMINESCENCE

The use of spectrofluorimetry to analyze the (n,m) distribution of semiconducting species in single-walled carbon nanotube (SWNT) samples has been increasingly widespread.\textsuperscript{25,41,44-57} Nanotube fluorescence in the near infrared wavelengths is advantageous due to low background signal and the dearth of interfering emitters in this spectral region.\textsuperscript{25} Only semiconducting nanotubes of the first van Hove transition (E\textsubscript{11}) are visible, however.\textsuperscript{22} Nanotubes must also be solubilized, or grown in an isolated manner, in order to produce a signal, as orthogonal electronic dispersion prevents bundled SWNT from fluorescing.\textsuperscript{44}
Fluorescence excitation profiles allegedly show the relative concentrations of the semiconducting nanotubes in a SWNT solution. For this reason, fluorescence is used to gauge the distribution of nanotube species produced by synthesis techniques including HiPco, CoMoCAT, and alcohol catalytic CVD. We have recently shown that nanotube quantum yield depends nonlinearly on length, however, and SWNT can be cut by the ultrasonication process used to disperse them in solution under some conditions. Thus, the use of fluorescence alone may not be a valid measure to properly assess the distribution of species in a sample. Furthermore, we have reported previously that ultrasonication does not cut all species uniformly, shortening larger diameter tubes to a greater extent than small diameters.

Recent reports have been ambiguous with regard to ultrasonic dispersion techniques of nanotubes. In this study, we process nanotubes under typical conditions, producing SWNT solutions with different optical properties. A fluorescence excitation profile of cholate-suspended SWNT, dispersed via a cup-horn sonicator without additional probe-tip ultrasonication, is shown in Figure 3.14 (top). The profile is similar to that of SWNT in sodium dodecyl sulfate (SDS), though the transitions are redshifted slightly due to the cholate surfactant. The (6,5) tube’s fluorescence signal is 58% less than that of the (8,4) nanotube, the strongest peak in the spectrum, and also lower than the (7,5), (7,6), and (8,6) nanotubes’ signals. Figure 3.14 (bottom) shows a fluorescence profile of the same HiPco SWNT suspended in cholate and probe-tip sonicated in a microcentrifuge tube at 3W for 10 minutes without a cooling bath. The ultrasonication causes considerable heating of the sample and reaches approximately 85 °C by the 10-minute mark. Total fluorescence signal decreases with probe-tip ultrasonication, though
not uniformly. The fluorescence of the (6,5) nanotube is over 30% higher than that of the (7,6) nanotube, the next highest peak. It is unclear whether previous researchers controlled temperature during ultrasonication and may have similarly changed the apparent distribution of the sample in this way.
Figure 3.14: (Top) Fluorescence excitation profile of sodium cholate-suspended SWNT in water. (Bottom) Profile of the same SWNT after 10 minutes of probe-tip ultrasonication conducted without controlling temperature. This result is similar to profiles shown by other researchers as evidence for selective synthesis.\textsuperscript{41,44}
We find that concurrent heating during ultrasonic processing allows us to control the degree of spectral change. We study this by looking parametrically at temperature during processing. Increasing the temperature during probe-tip ultrasonication causes a decrease in the total fluorescence signal as well as relative changes in the intensities of certain species (Figure 3.15). As seen in the profile, when the temperature is increased, the (6,5) nanotube fluorescence attenuates to a lesser degree than all other species. In the starting material, the (6,5) and (7,5) signals are of similar intensity, and the (8,4) nanotube’s signal is the strongest. However, in the sample probe-tip sonicated at 80 °C, the (6,5) nanotube has the strongest signal, and the (7,5) fluorescence signal has decreased to a much greater extent than the (6,5).

Figure 3.15: Fluorescence spectra at 554 nm excitation of sodium cholate-suspended nanotubes probe-tip sonicated at different temperatures compared to a control sample which was not sonicated using a probe-tip. The 80 °C curve is shown 4 times its actual intensity.
Although the shortening of nanotubes, caused by ultrasonication, can lead to an anisotropic decrease in quantum yield of a nanotube sample, the observed changes in fluorescence intensity at different temperatures could also result from sonochemistry performed at the nanotube surface. This is likely because the decrease in fluorescence does not appear to follow the diameter-dependent trend seen in earlier cutting work \(^{45}\) and because absorption spectra of tubes sonicated at elevated temperatures show altered peak-to-valley ratios compared to the original HiPco tubes (data not shown). Also, probe tip ultrasonication of nanotube samples for long periods (as long as 10 hours, though in batches of 5 ml instead of 400 \(\mu\)L) in an ice water bath does not lead to the observed changes.

The large differences in fluorescence, caused by small variations in ultrasonic processing conditions of the same sample of nanotubes, are cause for concern to researchers using fluorescence alone to benchmark carbon nanotube synthesis methods. Ultrasonication, necessary for dispersing nanotubes, may cause changes in relative intensities of SWNT species, leading to erroneous conclusions. Additional analytical methods, such as absorption spectroscopy, should be employed. We urge researchers to consider these processing-related effects and to fully document the conditions employed (ultrasonicator tip geometry, power, cooling method if any, etc.) in future scientific publications.

3.6 RAMAN SPECTRAL BENCHMARKING OF SWNT AGGREGATION

Systematic changes in the Raman spectrum are correlated herein to variations in sample morphology characterized by microscopy. In Figure 3.16, four different HiPco
SWNT samples are compared at 785 nm excitation using the RBM region. In plate a, we show the material dispersed in 1% SDS at pH 10. This material was flocculated from solution to produce Figure 3.16b. This is compared to material used as received with 50% Fe catalyst by weight and unprocessed after reactor synthesis (Figure 3.16c). Finally, if the material in Figure 3.16a is precipitated from solution via water evaporation, the spectrum in Figure 3.16d is obtained. Graphics beside the spectra depict the believed aggregation state of the material which generally increases from Figure 3.16a to 3.16d. Scanning and cryo-transmission electron micrographs of the samples show evidence of this aggregation. A cryo-TEM of nanotubes suspended in SDS, Figure 3.16a, shows individual nanotubes dispersed in solution displaying minimal inter-tube contact. The flocculated nanotubes, Figure 3.16b, associate into mats of loosely-aggregated nanotubes. Raw HiPco SWNT that have not been previously dispersed exhibit wound bundles, mats, and various other morphologies (Figure 3.16c). The nanotubes precipitated from SDS, Figure 3.16d, form tightly roped bundles. The spectral comparison reveals substantial diminution of the prominent mode at 234 cm\(^{-1}\) and an increase in one at 267 cm\(^{-1}\). Previous work shows that the latter increases absolutely, not merely relative to other modes.\(^{31}\) The significant variability in relative intensities in this region is echoed at other Raman excitation wavelengths and raises concerns about using this region exclusively to make statements about sample composition.

As nanotubes aggregate into parallel, aligned contact, their \(k_z\) band structure is altered and they develop an orthogonal electronic dispersion to their otherwise 1-D axial dispersion along the tube length. Reich and co-workers have accurately modeled this process using ab-initio calculations.\(^{59}\) In practice, the absorption spectrum of nanotube
bundles is red shifted and broadened compared with pristine nanotubes.\textsuperscript{25,31} These changes to the absorption line-shape affect the Raman spectrum in several ways. Most notably, a species may be selectively brought into resonance resulting in an increase in the overall scattering intensity. The converse is also possible. As an aside, note that within the resolution of the spectrometer used in this work (4 cm\textsuperscript{-1}), there is no shifting of the RBM peak center with aggregation as anticipated by experimental high pressure studies and molecular dynamics simulations.\textsuperscript{60}
Figure 3.16: Raman spectra taken at 785 nm with scanning electron micrographs and represented aggregation states for four samples prepared with different morphologies. The RBM regions are shown for a sample dispersed in aqueous solution using 1% wt. sodium dodecyl sulfate at pH 10 (a). HiPco nanotubes flocculated from solution with acetone and washed in methanol (b). HiPco SWNT used as received (c). Nanotubes precipitated on a glass slide from SDS solution and washed to remove surfactant (d).
Some insight is gained upon closer examination of the data in Figure 3.16. We have modeled this process at 785 nm in detail using the spectral assignment of the nanotubes in Figure 3.16. We assume a simple, Gaussian distribution in nanotube diameter $d_n$, ($\sigma = 0.2$ nm, $d_{\text{mean}} = 0.93$ nm) for the relative intensities of the highly dispersed material. Figure 3.17 shows the location of six $(n,m)$ nanotubes within the assumed distribution. Describing each feature as a Lorentzian line-shape in the Raman spectrum with shift given by $223.5/d_n + 25.5$, the Raman intensity, $I(E_{\text{Laser}})$, of each feature is described assuming that the corresponding inter-band transition can be simplified as a delta function at energy $E(n,m)$.\(^{61}\)

\[
I(E_{\text{Laser}}) \propto \frac{1}{\sigma \sqrt{4\pi}} \exp \left[ -\frac{(d_n - d_{\text{mean}})^2}{4\sigma^2} \right] \left( \frac{E_{\text{Laser}} - E_{(n,m)} + \Delta E}{\Gamma} \right)^2 - \frac{\Gamma^2}{4} \left( \frac{E_{\text{Laser}} - E_{\text{phonon}} - E_{(n,m)} + \Delta E}{4} \right)^2 - \frac{\Gamma^2}{4}
\]

Here, $E_{\text{Laser}}$ is the excitation energy with $\Delta E$ and $\Gamma$ being the energy shift upon roping and peak broadening based on the particular physical or chemical environment of the nanotubes. Figure 3.18a-c presents the simulated Raman spectrum with increasing $\Gamma$ and $\Delta E$. Despite the simplicity of the assumptions, the model qualitatively describes the spectral changes observed experimentally in Figure 3.16. An examination of the simulated excitation profiles based on the above expression provides some insight into the observed spectral changes (Figure 3.18d-f). The mode at 267 cm$^{-1}$ is the $(10,2)$ nanotube that is initially off resonance in individually dispersed material. With aggregation, as transitions shift to lower energy, this feature is brought into resonance as others move away. Note that we have assumed with no justification that all transitions shift by the same amount $\Delta E$. The magnitude of this constant shift is consistent with
what is observed in the absorption spectrum\textsuperscript{25} of roped material experimentally (~60 meV.) The change in the line-shape, $\Gamma$, is also well within what is expected for nanotubes in bundles, as values as high as 120 meV have been reported for experimentally measured excitation profiles.\textsuperscript{62}

\textbf{Figure 3.17}: Estimated concentration distribution of single walled carbon nanotube diameters of the 6 HiPco nanotubes present in the 785 nm Raman spectrum
Figure 3.18: Simulated Raman spectrum at 785 nm excitation as a summation of Lorentzians with a deviation of 7 (cm$^{-1}$)$^2$ using intensities calculated from two factors: the simplified excitation profile represented as equation 1 and the diameter dependent concentration distribution in Figure 3.17. Spectra are parametric in relative energy shift $\Delta E$ and broadening $\Gamma$ (in equation 1.) For a) $\Delta E = 0$, $\Gamma = 40$ meV, b) $\Delta E = 20$, $\Gamma = 60$ meV, c) $\Delta E = 60$, $\Gamma = 80$ meV; the corresponding composite excitation profiles are d); e) and f) respectively.

Similar aggregation effects are noted at other Raman wavelengths. We include experimental data at 514.5 nm excitation as this wavelength is frequently used to probe metallic nanotubes for HiPco samples. In this case, the $v_3 \rightarrow c_3$ transitions of semi-conducting nanotubes are initially off resonance (Figure 3.19a), while aggregation brings...
these features into resonance as observed previously. As a result, the intensity of the feature at 190 cm\(^{-1}\) attributed to semi-conductors is sensitive to the aggregation state of the sample, as are high frequency modes in this region as shown (Figure 3.19b). In this case, however, several semi-conducting modes appear convoluted, and the shift is merely a change in the excitation window. We cannot apply the same analysis, as no assignment for v3→c3 yet exists.

![Figure 3.19: Raman spectra at 514.5 nm excitation for samples prepared with different morphological properties. The RBM regions are shown for a sample dispersed in aqueous solution using 1% wt. sodium dodecyl sulfate at pH 10 (a). The solid sample is HiPco SWNT precipitated on a hot plate from SDS solution (b).](image)

Changes in the tangential mode region of the Raman spectrum also complicate its utility in gauging changes in metallic and semi-conductor concentration. As described earlier, this mode splits into two components due to differences in the force constants for the carbon-carbon vibration for bonds axial and circumferential to the tube. The latter is
able to couple to the continuum of electronic transitions near the Fermi level for metallic nanotubes exclusively and the result is a BWF line-shape.\textsuperscript{28} This line-shape is frequently used to identify the presence of metallic nanotubes in the sample for this reason. However, it follows that this feature should also be sensitive to processes that affect the electronic continuum. This is in fact the case experimentally (Figure 3.20). We show in earlier work that this BWF feature can shift to higher wavenumbers and effectively become indistinguishable from the Lorentzian component with increasing H\textsuperscript{+} concentration\textsuperscript{30} in solution. Metallic nanotubes in bundles are known to undergo electronic perturbations including the opening of a pseudo-gap as documented earlier in both theoretical\textsuperscript{59} and experimental work.\textsuperscript{63} We underscore that aggregation and bundling of nanotubes causes changes to this BWF region.

**Figure 3.20:** Four samples prepared with different morphological and chemical properties for Raman spectroscopic characterization at 514.5 nm excitation. The TM regions are shown for two samples are dispersed in aqueous solution using 1% wt. sodium dodecyl sulfate at pH 10 (a) and pH 2 (b). Two solid samples are HiPco SWNT used as received (c) and rapidly precipitated on a hot plate from SDS solution (d).
The orthogonal electronic dispersion that develops as a result inter-tube electrical contact broadens and shifts the inter-band transitions to lower energy. We have shown that the magnitude of this shift depends upon the extent of bundle organization and inter-nanotube contact area. Evidently, aggregation shifts the effective excitation profile and causes peaks to increase or decrease depending upon where the transition lies relative to the excitation wavelength in the Raman spectrum. We modeled the result using a simplified δ-function representation of nanotube electronic structure. The findings are particularly relevant for evaluating nanotube separation processes, where relative peak changes in the Raman spectrum can be confused for selective enrichment. This correlation should also assist in the development of highly dispersed composite materials.

3.7 EXPERIMENTAL DETAILS

3.7.1 Nanotube Suspension

Single-walled carbon nanotubes produced by the HiPco method\textsuperscript{64} were obtained from Rice University. Nanotubes were suspended in 100 mM sodium cholate hydrate (Sigma) or a 1% solution of sodium dodecyl hydrate (SDS) by cup-horn sonicating for ten minutes and centrifugating for 4 hours at 30,000 rpm.\textsuperscript{25}

3.7.2 Ultrasonication Studies

To cut SWNT by ultrasonication, the suspensions were processed for 1 to 10 hours at 45% amplitude (3 watts) using a Sonics VC 130 probe-tip sonicator (Vibra Cell) with a 2 mm tip in a cooling cell apparatus (Vibra Cell). For sonochemistry studies, nanotube suspensions were processed for 10 minutes in batches of 400 μL at a time in a
microcentrifuge tube without cooling. Temperature control was accomplished by sonicating the sample in an oil bath on a temperature-regulated hotplate.

3.7.3 Electrophoresis and Chromatography

Electrophoresis was performed in a 7 x 10 cm, 1% agarose gel in TAE buffer (Tris-Acetate-EDTA) with 50 mM sodium cholate at 100 V. Nanotube fractions were removed from the gel via electroelution by creating a second set of 8 wells in the gel 4.5 cm from the original 40 μL sample wells. Material was pipetted out of the second set of wells after 30 minutes of electrophoresis and repeatedly after 5 additional minutes of applied potential to obtain 6 fractions. Gravity flow size exclusion chromatography was performed with Sephacryl S-500 gel filtration chromatography media (Amersham Biosciences) in a 100 cm Kontes FlexColumn Economy Column (Fischer) with 1.5 cm internal diameter using TAE buffer with 50 mM sodium cholate.

3.7.4 Aggregated SWNT Sample Preparation

Acetone was added to flocculate and remove nanotubes from this solution. The flocculent mass was then washed with methanol to remove excess surfactant. Nanotubes were precipitated from the SDS solution by heating and evaporating the liquid on a glass microscope slide. Dried nanotubes were washed in methanol to remove SDS.

3.7.5 Spectroscopy

Raman spectroscopy at 514.5 nm was performed using a SPEX 1877 Triplemate triple grating monochromator and a Princeton Instruments SPEC-10 400B Digital CCD
camera. Raman spectra with 633 nm excitation were taken with a LabRam-IR (Jobin Yvon Horiba) and at 785 nm using a Kaiser Optical Holospec f/1.8 imaging spectrograph with a fiber optic probe head incorporating both collection optics and excitation laser aperture. This spectrometer also measured fluorescence peaks to approximately 1080 nm. Raman/fluorescence spectra were taken on electrophoresis gels by aiming the probe head 90° into the agarose gel. The gels were held on an automated x-y translation stage and scanned 0.5 mm between every spectrum taken. This produced spatially-parsed sets of Raman spectra over the length of the gel. Spectra were processed via the Kaiser Holoreact program for Matlab (The Mathworks, Inc.). Peak heights of all Raman and fluorescence features were calculated at defined wavelength intervals over each spectrum in the gel. A Shimadzu UV-3101PC UV-VIS-NIR Scanning Spectrophotometer was used for absorption spectra. Fluorescence spectroscopy measurements, including 3D profiles, between 900 and 1400 nm were conducted with a spectrofluorimeter built in-house and a liquid nitrogen-cooled Edinburgh Instruments EI-L Ge detector.

3.7.6 Microscopy

AFM samples were prepared by depositing nanotube solutions onto silicon wafers coated with 3-aminopropyltriethoxysilane (APTES) and rinsed with water. Tapping mode AFM images were taken with a Digital Instruments Dimension 3100 with BS-Tap300Al silicon probes (Budget Sensors). Scanning electron microscopy (SEM) was performed by depositing nanotubes on a silicon wafer and imaging with a Hitachi S4700. Cryo-transmission electron microscopy (cryo-TEM) measurements were conducted as described in Moore, V. et. al.\textsuperscript{58}. 

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35. We reject the possibility of aggregation causing spectral changes here because nanotube fluorescence disappears during aggregation.


4.1 INTRODUCTION AND BACKGROUND

The electronic and optical properties of single-walled carbon nanotubes (SWNT) have inspired their incorporation into new structural,1-3 microelectronics,4,5 and sensing applications.6,7 A need exists for robust cellular markers that will not: (1) have untoward effects on nuclear or cell membrane function, (2) impact cellular viability during long-term studies, (3) quench after cytochemical staining,8 or (4) blink9 or photobleach10 under prolonged excitation. Carbon nanotubes are promising agents for solving these cell labeling problems due to their optical properties. Nanotube optical emission can also be modified by the tube’s environment and thus used as a biosensor.11,12 We explore the possibility of developing this application within mammalian cells in order to extend SWNT optical sensors that we recently implemented on the sub-cellular level.6

The introduction of nanotubes into eukaryotic cells has been investigated by several researchers. The majority of investigations have been concerned with drug or gene delivery where SWNT, suspended by a biomolecule, was tagged with a fluorophore for observation.13-15 Two of these studies13,14 used covalently functionalized SWNT, which exhibit greatly reduced Raman and absorption cross sections16 and quenched photoluminescence.17 One study18 used the band gap fluorescence of surfactant-suspended SWNT to assess the toxicity of nanotubes in macrophages. The study showed nanotube fluorescence in cells up to 24 hours after incubation, and the mechanism of intake was only applicable to cells which undergo phagocytosis.

In this chapter, carbon nanotubes encapsulated by a DNA oligonucliotide (DNA-SWNT) were used as photobleaching-resistant, non-quenching markers which remained
functional in live cells for up to 3 months. Raman scattering and fluorescence spectra of DNA-SWNT were measured from within live as well as hematoxylin and eosin (H&E) stained cells, showing continued emission after staining and spectral changes upon uptake. Transmission electron microscopy (TEM) was compared to light microscopy to confirm peri-nuclear endosomal localization of the nanotube complexes. The potential of DNA-SWNT as long-term cellular biomarkers and sensors is assessed.

4.2 ADVANTAGEOUS OPTICAL PROPERTIES OF SWNT FOR BIOLOGICAL STUDIES

Current methods used to label cells\textsuperscript{19} and sub-cellular components\textsuperscript{20-23} are generally based on organic fluorophores such as fluorescein or members of the rhodamine, cyanine, or alexa chemical families.\textsuperscript{24,25} However, these fluorophores have several limitations which restrict their utility in applications such as cell proliferation, stem cell differentiation, and real-time microscopy experiments lasting multiple hours.\textsuperscript{22,26-31} Photobleaching of the chromophore limits the ability of investigators to observe their specimens repeatedly and over long periods.\textsuperscript{10} Organic fluorophores also quench when applied concurrently with H&E, an important stain used by pathologists and other researchers for evaluation of histological sections.\textsuperscript{8,32,33}

Quantum dots are highly luminescent semiconducting nanoparticles that overcome some of these limitations.\textsuperscript{34} They are approximately 100 times more resistant to photobleaching than organic fluorophores, however their signal attenuates significantly under prolonged excitation. They have a limited lifetime in aqueous solutions (approximately 7-12 days), and their cores, often composed of cytotoxic cadmium selenide, can be difficult to shield from the cellular medium.\textsuperscript{35,36}
Single-walled carbon nanotubes (SWNT) are composed of cylindrical graphene approximately 0.6 – 2 nm in diameter. The electronic structure and diameter of carbon nanotubes determine the spectral characteristics seen in absorption, fluorescence, and Raman scattering. Individually dispersed nanotubes are required for absorption and fluorescence. This is accomplished by surfactants, polymers, proteins, and DNA encapsulation. Nanotubes exhibit strong Raman scattering due to resonance enhancement at SWNT absorption transitions. The 1-D structure of nanotubes results in sharp interband transitions of SWNT absorption spectra, as well as photoluminescence of semiconducting nanotubes in the near-infrared region (800-1600 nm). These wavelengths include the tissue-transparent region of the electromagnetic spectrum (800-1400 nm), in which radiation passes through live cells without significant scattering, absorption, heating, or damage to tissues.

Raman spectroscopy is increasing in popularity with life scientists. Raman scattering normally produces small intensities of Stokes and anti-Stokes shifted light from a molecule that exhibits anisotropic polarizability. Nanotubes, however, produce strong, resonance enhanced Raman bands 150 – 300 cm\(^{-1}\) and 1590 – 1600 cm\(^{-1}\) away from the excitation wavelength. The former, called the radial breathing modes (RBMs), are caused by uniaxial vibrations, and the latter, called the tangential mode (G peak), is caused by stretching along C-C bonds of graphene. Both metallic and semiconducting SWNT demonstrate intense Raman scattering with some of the highest known cross sections for single molecules (at 5.7x10\(^{-21}\) cm\(^{2}\)/sr/molecule for a 1 micron nanotube excited at 785 nm). The scattering exhibits resonance enhancement when incident light coincides with an optical transition of the nanotube. Due to the high
Raman scattering cross-section of SWNT and resonance enhancement at near-infrared absorption transitions, Raman scattering of nanotubes is easily detectable, unmistakable, and often of similar intensity as fluorescence events. Furthermore, it does not blink or quench and will not diminish under prolonged excitation.\textsuperscript{37,48,52,53}

Carbon nanotube fluorescence is extremely photostable, showing no evidence of blinking or photobleaching after prolonged exposure to excitation at high fluence.\textsuperscript{18} Figure 4.1 shows the rate of photobleaching of an organic fluorophore, quantum dots, and carbon nanotubes. The fluorescence emission of DNA-SWNT, with excitation at a fluence of 1.3 MW/cm\textsuperscript{2} for 10 hours, showed no attenuation over the entire duration. This is compared to photobleaching of near-infrared quantum dots, consisting of a CdTe core and CdSe shell, and the near-infrared organic fluorophore 78-CA, both excited at 600 mW/cm\textsuperscript{2}.\textsuperscript{54} The organic dye emission decreased to 20\% of its original intensity in 24 seconds of excitation, while the quantum dots decreased to 95\% of their original intensity within 30 minutes and to 43\% in 10 hours.\textsuperscript{54,55} Like its fluorescence emission, carbon nanotube Raman scattering remained constant during continuous excitation (data not shown). Therefore, both nanotube Raman scattering and fluorescence can be measured and tracked over long durations without signal attenuation. The quantum yield of fluorescence for semiconducting carbon nanotubes was initially estimated at 0.1\%,\textsuperscript{17,56} however recent studies suggest the true value for pristine nanotubes is larger and strongly length-dependent.\textsuperscript{57} Fluorescence emission in the near-infrared region, exclusive to few moieties including carbon nanotubes, has strong advantages over visible fluorescence. Living tissues and other biological media are transparent in the near-infrared spectral region.\textsuperscript{58} Human blood, for instance, has a narrow window from 900 to 1400 nm where
light can penetrate from 3 – 5 cm.\textsuperscript{58} Few conventional markers absorb or emit strongly in this region, and those that do are often photochemically unstable.\textsuperscript{59} The amount of collected light $I$ from an optical probe with quantum yield $\phi$ placed in a medium with absorbance $\mu(\lambda)$ at $\delta$ distance away from a surface illuminated at intensity $I_o$ is roughly

$$\ln\left(\frac{I}{I_o}\right) = \ln(\phi) - 2\mu\delta$$

in a 1-D approximation. At a 1 cm depth into oxygenated blood, the second term is 800 at 550 nm (visible fluorophore), 20 at 900 nm (near-infrared quantum dots) and only 0.8 at 1200 nm (a species of carbon nanotube). In contrast, there is a much weaker dependence on $\ln(\phi)$, which varies only by 6.9 between $\phi = 0.001$ to 1.

Few biological systems naturally fluoresce in the near-infrared, while cellular pigments and inclusions (E.G. lipofuscin and hemosiderin) are usually strongly fluorescent in the visible region. This background or auto-fluorescence of biomolecules is a pervasive feature that limits the sensitivity and selectivity of fluorescence-based cell labeling methods and is the central limitation in single-molecule methods. Carbon nanotubes remove this obstacle because of their near-infrared emission and scattering.
Figure 4.1: Spectral characteristics of DNA-SWNT. (a) Photobleaching comparison for organic, quantum dot, and SWNT fluorescence. The organic near-infrared dye 78-CA and near-infrared CdTe/CdSe quantum dots were excited at 600 mW/cm² fluence and carbon nanotubes were excited at 1.3 MW/cm². The latter (magnified at right) show no bleaching over 10 hours.

4.3 MICROSCOPY AND SPECTROSCOPY OF CELL-BOUND SWNT

Single-walled carbon nanotubes, synthesized by the HiPco method, and suspended in water with an alternating G-T sequence of single-stranded DNA, show strong Raman scattering and near-infrared fluorescence emission upon exciting at 785 nm (Figure 4.2, curve 1). The SWNT Raman radial breathing modes (RBMs) between 150 – 300 cm⁻¹ show only a minute 267 cm⁻¹ peak, which suggests that nanotubes are individually suspended. The tangential Raman mode, characteristic of graphite and its derivatives, appears at 1598 cm⁻¹. This is seen in any Raman spectrum containing nanotubes which have not been heavily chemically derivatized. One small and two large fluorescence peaks are visible at 2023, 2697, and 3172 cm⁻¹ (corresponding to 933, 996, and 1045 nm respectively). This spectrum suggests a well-dispersed solution containing individual, near-infrared fluorescent nanotubes. Additional larger nanotube fluorescence peaks appear at longer wavelengths and are detectable with near-infrared sensitive
photomultiplier tubes, germanium and InGaAs detectors. Encapsulation by the G-T
ssDNA oligonucleotide ensures stability in laboratory conditions on the order of months
in aqueous solutions without the use of a surfactant.\textsuperscript{42-44} The DNA-SWNT complex is
also stable upon exposure to nucleases, various ionic strengths (data not shown), and pH
between 8 and 2.5.\textsuperscript{42,61}

Upon addition to the cell media, DNA-SWNT exhibited red-shifted fluorescence
emission and a lower fluorescence/Raman tangential mode ratio relative to the starting
material (Figure 4.2, curve 2). Murine myoblast stem cells and 3T3 cells in media
incubated with DNA-SWNT for periods ranging from 1 to 48 hours showed a red shift of
30–40 cm\(^{-1}\) in the nanotube spectra relative to the starting DNA-SWNT spectrum. A
representative spectrum is shown in Figure 4.2, curve 3, although slight variations
occurred across samples (Figure 4.2). The shift and relative decrease in fluorescence of
nanotubes upon entering the cell is believed to be caused by the acidic environment in the
vicinity of the nanotube-DNA complex.\textsuperscript{11} These changes will be the subject of a
subsequent manuscript.
Figure 4.2: Combined Raman and fluorescence spectra, at 785 nm excitation, of single-walled carbon nanotubes encapsulated by single-stranded DNA (1) suspended in water, (2) in cell media, (3) in a murine 3T3 cell. The Raman modes (bottom inset) at 150 – 300 cm\(^{-1}\) and 1598 cm\(^{-1}\) tangential mode are visible along with near-infrared fluorescence peaks (top inset) at 2023, 2697, and 3172 cm\(^{-1}\). Spectra were scaled to equalize Raman peak intensities.

Area maps, compiled from multiple Raman spectra of live murine 3T3 and myoblast stem cells, incubated with DNA-SWNT and left in culture for 48 hours and 8 days, show that the nanotubes concentrated near, but outside, the nuclei of the cells.

Figure 4.3 maps the intensity of the Raman scattering from 150 cm\(^{-1}\) to 300 cm\(^{-1}\) over a 80 x 85 \(\mu\)m area of the 3T3 cell culture overlayed onto a digital image of the region from which the spectra were taken. From the area map compilation, we find interspersed areas of elevated Raman intensity, which correspond to regions of higher SWNT concentration. Similar results are found in myoblast stem cells. Spectra taken at specific points from the area maps include the Raman radial breathing modes, tangential mode, “G-prime” peak, and fluorescence of carbon nanotubes.
Figure 4.3: Spectra and corresponding Raman intensity area maps of live 3T3 fibroblast and myoblast stem cells. (a) Combined Raman and fluorescence spectra of live murine 3T3 cells incubated with DNA-suspended SWNT. The three spectra correspond to locations on the area map. Absence of a 267 cm$^{-1}$ Raman peak suggests minimal SWNT-SWNT contact (inset). (b) Area map of Raman RBM intensity of nanotubes in live 3T3 cells after 48 hours in culture overlayed onto an optical micrograph of the same region. (c) Raman and fluorescence spectra of live murine myoblast stem cells incubated with DNA-suspended SWNT. The three spectra correspond to locations on the area map. (d) Area map of Raman RBM intensity of nanotubes in live myoblast stem cells after 48 hours in culture overlayed onto an optical micrograph of the same region. (e) Raman and fluorescence spectra of nanotubes in live 3T3 cells after 8 days in culture. (f) Area map of RBM intensity of 3T3 cells in culture for 8 days. The scale bars represent 20 μm. Color legend is valid for all images.
After 8 days in culture (Figure 4.3, bottom), nanotube spectra in 3T3 cells showed a marked decrease in the fluorescence relative to the Raman scattering, although nanotubes continued to exhibit both types of emission and exhibit localization in the perinuclear region of the fibroblasts. Nanotube fluorescence also exhibited spectral variations, evident in the shape of the 2697 cm$^{-1}$ peak, which we can attribute partially to the acidic environment surrounding the nanotubes, although other endosomal-bound proteins and degradation of the DNA coating may also contribute to these changes.

Peri-nuclear accumulation of DNA-SWNT within membrane-enclosed vesicles was confirmed by TEM. Micrographs (Figure 4.4) show that the carbon nanotubes formed uniaxial, ordered bundles inside vesicles located near the nucleus, but DNA-SWNT were not found within the nuclear envelope. This lack of distribution of DNA-SWNT within the nucleus appeared to be a characteristic feature of all micrographs taken with these samples. It is possible that single, non-aggregated carbon nanotubes could have localized within the nucleus. However, deliberate visual observation of the nuclear area performed at a magnification (50,000x) that could easily detect single DNA-SWNT complexes in the cytoplasm did not reveal carbon nanotubes within the nucleus. These findings appear to suggest an endocytotic transport mechanism of fluorescent DNA-SWNT aggregates into cytoplasm-bound vesicles without penetration of the DNA-SWNT complexes into the nuclear envelope.
Figure 4.4: TEM images of carbon nanotubes in murine myoblast stem cells. (a) Whole cell image of a murine myoblast stem cell containing vesicle-bound DNA-SWNT. (b) Micrograph of boxed region in (a) showing 2 peri-nuclear inclusion bodies containing aggregated SWNT. (c) Membrane-bound vesicle-enclosed nanotubes. (d) A 50,000X-magnified vesicle from boxed region of (c) containing an aggregate of carbon nanotubes. Ordered stacking within the SWNT bundle is evident. The scale bars represent 1 μm in all images.

The H&E stain, used for standard histological analysis, is a mixture containing a large number of quenching agents: acetic acid, aluminium sulphates, haematein and mercuric oxides which colors acidic components of the cell, such as RNA and DNA, a blue color, and gives basic components a pinkish hue. Organic fluorophores and semiconductor nanoparticles do not emit through the dye. After applying the H&E stain to cells incubated with DNA-SWNT, strong nanotube Raman scattering peaks were still
visible in the spectra (Figure 4.5). In stained cells, the Raman radial breathing modes of nanotubes showed a relative increase of the 267 cm\(^{-1}\) peak and a decrease in fluorescence. An increase in the 267 cm\(^{-1}\) mode implies a shift in the interband transitions. When paired with a concomitant decrease of fluorescence, this change may imply direct nanotube-nanotube contact.\(^{60}\) Although the fluorescence disappeared, suggesting degradation or removal of the DNA coating, the persistence of biomarker emission (in the form of a Raman signal) represents a significant difference from organic fluorophores and semiconductor quantum dots used in cell labeling.

**Figure 4.5:** Raman spectra and area map of fixed, H&E stained murine myoblast stem cells. (a) Raman spectra including the radial breathing modes (RBMs), tangential mode, and G-prime peak. Numbered spectra correspond to locations from which they were taken. The RBM region exhibits an increase in the 267 cm\(^{-1}\) peak (inset) compared to the
same modes in spectra of unstained cells. (b) Area map of the RBM region signal intensity overlayed onto an optical micrograph of the same area. Similar spectra were obtained for the identical experiment performed using 3T3 cells. The scale bar represents 40 μm.

Long-term labeling capability that preserves cell viability was demonstrated by an experiment in which murine myoblast stem cells prepared identically to those used above were kept in culture for an additional 3 months. The cells reached confluence within two weeks of initial seeding and were maintained with weekly media changes. Cells were harvested from the flask and transferred to 4-well glass slides 3 days before spectroscopic measurements were taken. The optical micrograph and spectrum in Figure 4.6 were taken after H&E staining. The DNA-SWNT Raman RBMs and tangential mode were clearly visible and comparable to those taken from cells after 48 hours and 8 days in culture. Cell viability in long term culture appeared unhindered by the presence of nanotubes.
Figure 4.6: Murine myoblast containing carbon nanotubes after three months of incubation. (a) Bright-field image of H&E stained myoblast. Crosshair is centered on point at which Raman spectrum was taken. (b) Raman spectrum of the myoblast showing carbon nanotube radial breathing modes and tangential mode.

Nucleic acid-encapsulated single-walled carbon nanotubes show persistent Raman scattering and changing fluorescence spectra within live mammalian cells, functioning as cell markers for up to 3 months in culture. Nanotube Raman scattering could be seen through an H&E stain and remained visible inside cells for the duration of the experiment. Nanotube fluorescence, which does not photobleach even under prolonged excitation, remained visible after a week in live cells and exhibited wavelength shifts that responded
to the SWNT’s environment. Nanotubes were found to incorporate into cytoplasmic vesicles and labeled the peri-nuclear region of the cells but did not enter the nuclear envelope. Localization of the DNA-SWNT aggregate-containing vesicles suggests that these nanotubes follow an endocytotic pathway. The aggregates remained in the cells upon repeated cell divisions according to long-term experiments. Nanotubes will thus open the possibility of new experiments concerning cell proliferation and stem cell differentiation, long-term labeling of cell populations, and continuous monitoring of the nanotube environment within vesicles. Spectral changes in nanotube fluorescence will be utilized in our future work to fabricate long-term, optical sensors in vitro and in vivo.

4.4 EXPERIMENTAL DETAILS
Nanotubes were suspended with a 30-base alternating G-T sequence of single-stranded DNA (Biotechnology Center at the University of Illinois at Urbana-Champaign). Nanotubes and DNA were suspended in a 1:1 mass ratio in 0.1 M NaCl in distilled water and bath sonicated for 3 hours. The mixture was centrifuged (90 minutes at 16,000 g) and the pellet discarded.

The DNA-SWNT supernatant was added to 50% confluent cultures of murine 3T3 cells (CRL1658, ATCC, Manassas, VA) and murine myoblast stem cells (CRL 1772, ATCC, Manassas, VA) both in 5 ml of HEPES-buffered Dulbecco’s Minimal Essential Media (DMEM: Cellgro #15-018 CV; Mediatech, Herndon, VA) supplemented with 10% fetal bovine serum (FBS; Sigma) to create a final DNA-SWNT concentration of 0.22 mg/L. The cells were incubated under standard tissue culture conditions in the presence
of DNA-SWNT for time periods ranging from 1 to 48 hours and rinsed three times in fresh media before imaging or storing in long-term culture.

Raman and fluorescence spectra were taken with a Kaiser Optical Holospec f/1.8 imaging spectrograph attached to a Leica DMPL microscope. Myoblast stem cells left in culture for 3 months were fixed in methanol and stained with H&E before spectra were taken with a Renishaw inVia Raman microscope. Transmission electron microscopy studies were conducted using standard Karnovsky’s fixative/epoxy infiltration methods.64

Cells for electron microscopic evaluation were harvested using a solution of 0.5% trypsin/ 0.2% EDTA (Sigma) when cultures were approximately 80% confluent. The trypsin was neutralized by suspending the harvested cells in DMEM supplemented with an additional 10% FBS. The cells were then pelleted by gentle centrifugation (120xg for 10 min at 22°C). The media was washed from the cell pellet using Hanks Balanced Salt Solution (HBSS; Sigma) which was then replaced by Karnovsky’s fixative. The cell pellet was held in fixative at 4°C for 24 hr before epoxy infiltration and ultramicrotomy. Images were obtained using a Hitachi H600 electron microscope.

4.5 LIST OF REFERENCES


50. Estimated by comparison of an aqueous dispersion of HiPco SWNT at 10 mg/L with a cyclohexane standard where the absolute cross section has been measured.


55. The exact volume of fluid used for the photobleaching comparison was not listed in references used for Figure 4.1. We excited a 40 microliter volume with a 785 nm laser for our study of nanotubes.


63. Qtracker quantum dots with 655 nm peak emission (Quantum Dot Corporation) were incubated with murine myoblast stem cells and imaged with a fluorescence microscope. Quantum dots were visible within the cytoplasm of the cells. The myoblasts were then stained with H&E and re-imaged, showing no further quantum dot fluorescence. Quantum dots with 655 nm peak emission (Quantum Dot Corporation) were incubated with murine myoblast stem cells and imaged with a fluorescence microscope. Quantum dots were visible within the cytoplasm of the cells. The myoblasts were then stained with H&E and re-imaged, showing no further quantum dot fluorescence.

CHAPTER 5:
A SWNT SENSOR EMPLOYING SOLVATOCHROMISM AS THE DETECTION MECHANISM

5.1 INTRODUCTION AND BACKGROUND

The band gap energy of SWNT is sensitive to the local electronic environment around the nanotube, and this property can be exploited in chemical sensing, as was demonstrated for the detection of β-D-glucose. It has been shown that modulation of the local environment can result in changes to the wavelength of SWNT photoluminescence bands. We present herein the first SWNT-based optical sensor to employ shifts in emission wavelength.

Among the molecules that can bind to the surface of SWNT is DNA, which adsorbs as the right-handed B-form double-stranded (ds) complex. Certain DNA oligonucleotides will transition from the native, right-handed B form to the left-handed Z form as cations adsorb onto and screen the negatively charged backbone. We show herein that the B to Z transition for a 30-nucleotide dsDNA modulates the dielectric environment of the SWNT and decreases their near-IR emission energy up to 15 meV. We have used this fluorescence signal to detect divalent metal cations that bind to DNA and stabilize the Z form. The thermodynamics of the conformational change for DNA both on and off the SWNT are nearly identical. These near-IR ion sensors can operate in strongly scattering or absorbing media, which we demonstrate by detecting mercuric ions in whole blood, black ink, and living mammalian cells and tissues.
5.2 METALLIC CATION DETECTION VIA SWNT BAND SHIFTING RESPONSE TO DNA CONFORMATIONAL POLYMORPHISM

Near-IR spectrofluorometry was performed on colloidally stable complexes of DNA-encapsulated SWNT (DNA-SWNT) buffered at pH 7.4 and synthesized by non-covalent binding to the nanotube sidewall of a 30-base pair single-stranded DNA oligonucleotide with a repeating G-T sequence. Several types of semiconducting SWNT are present, but as we show below, they can be identified by their characteristic band gaps, and the shift in band gap is similar for each type of SWNT, although there is a diameter dependence. After addition of divalent cations, we observed an energy shift in the SWNT fluorescent emission with a relative ion sensitivity of Hg$^{2+} >$ Co$^{2+} >$ Ca$^{2+} >$ Mg$^{2+}$ which is identical for free DNA (Figure 5.1). The shift can also be observed by monitoring SWNT photo-absorption bands (Figure 5.2). The fluorescence peak energy traces a monotonic, two-state equilibrium profile with increasing ionic strength for each case {NOTE: Minor variability in the peak energy in the limit of zero concentration is caused by some batch-to-batch variation in the SWNT sample. The samples remain stable over long periods, although some additional shifting occurs when metal cations are present.}. 
Figure 5.1: Concentration-dependent fluorescence emission response of the DNA-encapsulated (6,5) nanotube to divalent chloride counterions. Inset shows the (6,5) fluorescence band at starting (blue) and final (pink) concentrations of Hg$^{2+}$.

Figure 5.2: The DNA-SWNT complex undergoes a red shift in absorption upon introducing HgCl$_2$. (a) Absorption spectra of the (7,5) nanotube with increasing concentrations of HgCl$_2$ added to a Tris buffered solution of DNA-SWNT. (b) Peak energy of the (7,5) nanotube versus HgCl$_2$ concentration.

Removal of the ions from the system via dialysis returns the emission energy to the initial value, which is indicative of a completely reversible thermodynamic transition (Figure 5.3)\textsuperscript{10}. Under the same conditions as the SWNT case, circular dichroism (CD)
spectroscopy confirmed that the unbound DNA strand undergoes a conformational change, upon introduction of Hg$^{2+}$, from the B to the Z form as the inversion of the 285 nm peak indicates a reversal of helicity (Figure 5.4).
**Figure 5.3:** Fluorescence energy of DNA-SWNT inside a dialysis membrane upon removal of Hg$^{2+}$ during a period of 7 hours by dialysis.

**Figure 5.4:** Circular dichroism spectra of unbound (dGdT)$_{15}$ DNA at various concentrations of Hg$^{2+}$. 
We compare the ellipticity of the 285 nm CD peak versus Hg$^{2+}$ concentration with the fluorescent emission energy from the nanotube under identical conditions (Figure 5.5). The overlapping points of inflection indicate that the difference in the free energy ($\Delta G$) changes for the DNA on and off the nanotube is quite small [$\Delta(\Delta G) \sim 0.05$ K$_b$T per phosphate, where K$_b$T is the thermal energy] $^{13, 14}$. Thus, the transitions for DNA in solution or adsorbed on the SWNT are apparently identical thermodynamically.

![Figure 5.5: DNA-SWNT fluorescence emission energy plotted versus Hg$^{2+}$ concentration (red curve) and the ellipticity of the 285 nm peak obtained via circular dichroism measurements upon addition of mercuric chloride to the same oligonucleotide (black curve). Arrows point to the axis used for the corresponding curve.](image)

A critical difference is apparent between slopes at the inflection, however. Pohl $^{15}$ describes the B-Z transition, which requires a double stranded helix to separate, change helicity and re-form, as a process of nucleation and propagation in series. The dsDNA strand initially separates with a ratio of rate constants $\beta_B/\beta_Z$ while propagation proceeds as a series of equilibrium steps proportional to the number of base-pairs, $N$, as the dislocation proceeds down the chain $^{10}$. The expression for the fractional transition $K^{6,15}$,
contains a scaling factor $C_0$ which is the ion concentration ($C$) where $K$ is independent of oligonucleotide length ($N$).

$$K = \left( \frac{C}{C_0} \right)^{aN} \left( \frac{\beta_B}{\beta_Z} + \left( \frac{C}{C_0} \right)^{aN} \right)^{-1}$$  \hspace{1cm} (5.1)

The slope at the inflection is related to the propagation length, $aN$. Regression of the data in Figure 5.5 reveals that DNA on the nanotube precedes through only 1/6 the number of transitions as in the case of the free strand. As expected, $\beta_B/\beta_Z$, which is associated with the initiation of the event, is similar for the cases on and off the nanotube (1.21 and 1.04 respectively). The model is one of a transition that propagates in small steps and requires about $2\pi/3$ radians of the strand to unravel for propagation down the nanotube (Figures 5.6-5.7).
Figure 5.6: Illustration of DNA undergoing a conformational transition from the B form (top) to the Z form (bottom) on a carbon nanotube.

Figure 5.7: Illustrations of DNA-encapsulated SWNT showing (at right) partial desorption of the oligonucleotide.
5.3 MODELING THE SWNT DIAMETER DEPENDENCE OF THE RESPONSE

Examining this phenomenon for SWNT of different diameters allows us to probe the influence of the cylindrical geometry. Perebeinos and co-workers\textsuperscript{16} used a numerical solution to the Bethe-Salpter equation\textsuperscript{17} to yield a scaling relationship for the exciton binding energy $E = A\mu^{n-1}r_t^{n-2}e^{-n}$ where $\mu$ is the reduced effective mass\textsuperscript{18}, $r_t$ is the nanotube radius, and $e$ is the dielectric constant around the nanotube. The constants $A$ and $n$ were determined by fitting nanotubes in the range of 1 to 2.5 nm and found to be 24.1 eV and 1.4 respectively. With this scaling, the change in emission energy from the B to Z form for a DNA wrapped nanotube is then:

$$\Delta E_{B\rightarrow Z} = A\mu^{n-1}r_t^{n-2}\left(\frac{1}{e_Z^n} - \frac{1}{e_B^n}\right)$$  \hspace{1cm} (5.2)

Approximating the dielectric constant of the B or Z wrapped nanotube using an effective medium:

$$e_i = \alpha_i e_{DNA} + (1 - \alpha_i)e_{Water}$$  \hspace{1cm} (5.3)

Here, $e_{DNA}$ and $e_{Water}$ are the dielectric constants of DNA (4.0) and water (88.1) and $\alpha_i$ is the ratio of surface area covered by DNA per total area which increases in transitioning from the B to Z form\textsuperscript{19}. To relate $\alpha_i$ to the geometry of the adsorbed phase, we consider a helical surface described by three parameters: radius, $r$, pitch, $b$, and width of the strand, $w$:

$$\alpha = \frac{w\sqrt{r^2 + b^2}}{rb}$$  \hspace{1cm} (5.4)

Describing the mechanics of DNA as a continuum helix\textsuperscript{20} maintaining an equilibrium curvature\textsuperscript{21}, one can describe the total energy in terms of its deflection. Adsorbing the
DNA to a nanotube of radius $r_i$ perturbs it from the equilibrium radius, $r_o$, and pitch, $b_o$.

The resulting pitch that minimizes the total energy is:

$$b = \sqrt{\left(\frac{r_o + r_i}{r_o}\right)(b_o^2 + r_o^2) - (r_o + r_i)^2}$$

and the surface area is:

$$\alpha = \frac{w}{(r_o + r_i)} \sqrt{\frac{b_o^2 + r_o^2}{b_o^2 + r_o^2 - (r_o + r_i)r_o}}$$

For B DNA, $r_o$ and $b_o$ are 1 and 3.32 nm respectively. For Z DNA the values are 0.9 and 4.56 nm$^{19,22}$.

We used fluorescence excitation profiles to examine the diameter dependence of the transition on the nanotubes experimentally. The emission from the B and Z forms (Figure 5.8, with vertical lines comparing the peak centers) shows that the (6,5) nanotube ($r = 0.38$ nm) undergoes a 15 meV decrease while the (8,7) ($r = 0.51$ nm) shifts only 5 meV. This inverse dependence for eleven of the strongest emitting SWNT in the sample is shown in Figure 5.9 and plotted in Figure 5.10.
Figure 5.8: Fluorescence 3D profile of excitation versus emission energy of a DNA-SWNT solution with 0 µM HgCl₂ (top) and 52,371 µM HgCl₂ (bottom) Vertical lines highlight the fluorescence red-shift of individual SWNT species upon Hg²⁺ addition.
Figure 5.9: Peak centers of the nanotubes present in the 3D profile.

Assuming these values and 0.51 and 1.18 nm for the regressed widths of the bands for B and Z respectively, the curve in Figure 5.10 is generated. Using constant dielectric values in equation 5.2 generates the horizontal line. Despite several limiting assumptions in this treatment \(^{23}\), the model is able to predict the correct magnitude of the energy shift, the trend with radius and direction of the shift (red) using only geometric constants for the DNA adsorbed phase.\(^{10}\)
Figure 5.10: The energy shift of individual SWNT species modified by the Bethe-Salpeter equation to evince the effective dielectric constant differences caused by DNA geometry (orange points). The model curve (blue line) is based on the radial dependence of the DNA surface area coverage of the nanotube on changing from the B to Z form.

5.4 DETECTION IN LIVE CELLS AND TISSUES

We have shown that the conformational rearrangement of a biomolecule can be transduced directly by the SWNT system. Given the recent discovery of a class of Z-DNA binding proteins, the association of Z-DNA to transcriptional activity and potential biological functions, it will be useful to have new probes to interrogate the conditions under-which Z-DNA formation can occur\textsuperscript{9,24}. Our previous work has shown that this type of DNA-SWNT complex is found to readily enter mammalian cells upon a 3-hour incubation and localize in the perinuclear region of the cell via endocytosis\textsuperscript{25}. In the present study, we localized DNA-SWNT within murine 3T3 fibroblasts (Figure 5.11) and perfused various concentrations of HgCl\textsubscript{2} (Figures 5.11 and 5.12)\textsuperscript{10} in the extracellular buffer space for 5 min. The SWNT emission from the (6,5) nanotube, although shifted
by 3 meV already upon uptake within the cell, red-shifts additively with increasing Hg$^{2+}$ concentration. After correcting for the initial shift caused by the new environment, the response of cell-bound DNA-SWNT fits the model curve created by the same complexes in pure buffer (Figure 5.12). Control experiments produce no additional shift. The successful operation of the complex within living mammalian cells creates opportunities for new molecular probes that operate in the n-IR and avoid natural autofluorescence of biological media.
Figure 5.11: (Top) Area map of the (6,5) nanotube peak fluorescence intensity of DNA-encapsulated SWNT within murine 3T3 fibroblast cells overlayed on an optical micrograph of the same region. (Bottom) Illustration of the experimental method used for ion binding response experiments conducted in mammalian cells. A cell containing endosome-bound DNA-SWNT undergoes 785 nm excitation through a microscope objective.
Figure 5.12: The (6,5) nanotube fluorescence peak energy of DNA-SWNT in 3T3 fibroblasts plotted versus Hg\textsuperscript{2+} concentration in the cell medium. The fluorescence energy of a population of 8 to 10 cells was averaged for each data point. Error bars show the first standard deviation. Blue line shows the model curve from original Hg\textsuperscript{2+} binding experiment conducted in Tris buffer. Inset shows individual spectra at each concentration.

Ion detection is also possible in media that already possess a strong ionic background. The introduction of a DNA-SWNT filled dialysis capillary to whole blood and mammalian tissue (Figure 5.13), and direct addition of nanotube complexes into a black dye solution (optical density > 4) still resulted in detection of HgCl\textsubscript{2} through these highly absorptive media (Figure 5.14). The near-IR fluorescence of DNA-SWNT in the dye solution exhibited the same response as SWNT in pure buffer. In whole blood and tissue, the presence of interfering absorbers of Hg\textsuperscript{2+} (free DNA, proteins, etc.) predictably shift the observed sensitivity to larger values, (C\textsubscript{0} = 3500 \mu M in blood; 8000 \mu M in tissue), however the DNA-SWNT still provide a measure of the residual ions that are locally bound to the complex in these heterogeneous media.
Figure 5.13: Dialysis capillary containing DNA-SWNT embedded in chicken tissue under 785 nm excitation through microscope objective.

Figure 5.14: The (6,5) nanotube fluorescence energy of DNA-SWNT in highly absorptive and scattering media: whole rooster blood (red triangles), black dye solution (black squares), and chicken tissue (green circles) plotted on a model curve from Hg$^{2+}$ addition to SWNT in buffer. The $\Delta E$ of all blood and tissue data points were corrected for an initial red-shift due to the environment.

5.5 EXPERIMENTAL DETAILS

5.5.1 DNA Encapsulation of Single-Walled Carbon Nanotubes

Single-walled carbon nanotubes synthesized by the HiPeo technique (Rice University) were suspended with a 30-base (dGdT) sequence of ssDNA (Biotechnology
Center at the University of Illinois at Urbana-Champaign) in a 1:1 mass ratio in 0.1 M NaCl in distilled water and bath sonicated for 3 hours. The mixture was centrifuged for 90 minutes at 16,300 g and the pellet discarded. The supernatant was then used for ion-binding response experiments. For ion binding response experiments conducted in Tris buffer, an additional dialysis step against distilled water was performed with 100,000 molecular weight cutoff dialysis tubing (Spectrum Laboratories Inc., Rancho Dominguez, CA) for 2-3 days.

5.5.2. Ion Binding Response of DNA-SWNT

DNA-encapsulated SWNT were diluted to concentrations between 2.4 mg/L and 3.9 mg/L buffered with 0.02 M Tris base adjusted to a pH of 7.4. Solutions of mercury(II) chloride (Aldrich), cobalt(II) chloride hexahydrate (Acros Organics), calcium chloride (Fisher), and magnesium chloride hexahydrate (EMD Chemicals Inc., Gibbstown, NJ) were introduced to the buffered DNA-SWNT and left to equilibrate at 22 °C for at least 2 hours. Spectrofluorimetry measurements were conducted using a Kaiser Optical Holospec f/1.8 imaging spectrograph (Kaiser Optical, Ann Arbor, MI). Circular dichroism measurements were taken with a JASCO J-715 spectrometer (JASCO International Co. Ltd., Tokyo, Japan) at room temperature. Fluorescence 3D excitation/emission profiles were acquired with a spectrofluorometer built in-house incorporating xenon arc lamp excitation, Kratos GM-252 monochromators (Kratos Analytical Inc., Chestnut Ridge, NY), and E1-L Germanium Detector (Edinburgh Instruments, Livingston, UK).
5.5.3 Removal of Mercuric Chloride

Mercury(II) chloride was removed from DNA-encapsulated nanotubes via dialysis against 3 L tris buffer. Dialysis tubing with 12,000 – 14,000 molecular weight cutoff (Spectrum Laboratories Inc., Rancho Dominguez, CA) was held in place in a beaker (Figure 5.15). Excitation (785 nm) and emission from the sample passed through the same probe head. Fluorescence was measured in real-time during dialysis.

![Experimental setup for real-time measurement of mercury(II) chloride removal. Dialysis tubing was held by clamps in a beaker. Excitation and emission light passed through the same probe head.](image)

**Figure 5.15:** Experimental setup for real-time measurement of mercury(II) chloride removal. Dialysis tubing was held by clamps in a beaker. Excitation and emission light passed through the same probe head.

5.5.4 Ion-Binding Response in Mammalian Cells

DNA-SWNT was added to 50% confluent cultures of murine 3T3 cells (CRL1658, ATCC, Manassas, VA) in 5 ml of HEPES-buffered Dulbecco’s Minimal Essential Media (Sigma) supplemented with 10% fetal bovine serum (Biomedia, Foster City, CA) to create a final DNA-SWNT concentration of 5 mg/L. The cells were incubated under standard tissue culture conditions in the presence of DNA-SWNT for 3 hours and removed from the culture flask by trypsin digest and plated onto a tissue culture dish. Optical and spectroscopic measurements were conducted with a Leica DMPL microscope (Leica Inc., Wetzlar, Germany) attached to the Kaiser Optical Holospec f/1.8 imaging spectrograph.
with 785 nm excitation at 100 mW. The excitation and collection light both passed through the microscope objective. Fluorescence area maps were compiled with multiple spectra taken via raster scanning.

5.5.5 Response in Highly Scattering Media

A dialysis capillary (Spectrum Laboratories Inc., Rancho Dominguez, CA) containing DNA-encapsulated nanotubes (323.76 mg/L) was placed in a vial of whole rooster blood containing the anti-coagulant heparin. Successive concentrations of mercury (II) chloride were introduced to the vial. Spectra were taken with 785 nm excitation at 400 mW. An identical capillary was inserted approximately 0.5 mm under the surface of chicken breast tissue (Figure 5.13). The tissue was incubated in mercury (II) chloride solutions for 3 hours before spectroscopic measurements were taken with 785 nm excitation at 100 mW. Black ink and DNA-encapsulated nanotubes (42.6 mg/L) were added directly to distilled water. Mercury(II) chloride was added to this mixture and spectra were captured with 785 nm excitation.

5.6 LIST OF REFERENCES


10. Materials, methods, and discussions of the modeling are available as supporting material on Science Online.


12. Minor variability in the peak energy in the limit of zero concentration is caused by some batch-to-batch variation in the SWNT sample. The samples remain stable over long periods, although some additional shifting occurs when metal cations are present.

13. The change in free energy difference is taken assuming equal propagation lengths as \( \Delta (D_{DG}) = kT \ln \left( \frac{C_f}{C_{SWNT}} \right) \) where \( C_f \) and \( C_{SWNT} \) are the free and SWNT bound DNA inflection concentrations respectively (from Figure 5.5). By comparison, the free energy change from the intramolecular contribution alone is 0.1 kT per phosphate. The \( \Delta (D_{DG}) \) normally has three contributions arising from hydration, electrostatic, and intermolecular terms.


23. The scaling developed by Perebeinos et al has several limitations, including an assumption that the dielectric constant is low, and the tube diameters under consideration are 1 nm or above.


CHAPTER 6:
MULTIPLEXED DETECTION AND IDENTIFICATION OF GENOTOXINS

6.1 INTRODUCTION AND BACKGROUND

Carbon nanotubes can be broadly functionalized and their 1D electronic structure is sensitive to molecular adsorption. The mechanisms of signal transduction include charge transfer interactions\(^1\), which alter the Fermi level of the nanotubes, and solvatochromic shifts\(^2\), which modify nanotubes’ intrinsic photoluminescence signatures, as we have shown\(^3\). The existence of multiple photoluminescent SWNT species suggests untapped potential for multi-modality as variations in their responses can be exploited to discern molecular properties and identify analytes within a mixture. In this work, we realize such a sensor defined by the differing responses of the (6,5) and (7,5) SWNT species. We detect and identify chemotherapeutic alkylating agents and reactive oxygen species (ROS) via unique spectral responses generated by their interaction with DNA-encapsulated nanotubes.

Active alkylating drugs and ROS are important biological analytes which are difficult to measure in vivo or in real time.\(^4\) Alkylating chemotherapeutic drugs from the nitrogen mustard and cis-platinum families are essential in treatments for multiple types of cancer. These drugs function by alkylating DNA, which leads to eventual strand breakage and results in apoptosis of mammalian cells.\(^5,6\) The agents degrade in the body within a few hours, impeding their measurement in live cells and tissues. Standard techniques, such as immunoassays, gel electrophoresis, and NMR\(^6,7\) typically cannot be performed in live cells or require preparation steps which inhibit real-time measurement. Nucleic acid damage due to ROS interaction with DNA is widely suspected to have a role
in oncogenesis and Alzheimer’s disease.\textsuperscript{8-10} Hydrogen peroxide, produced by mitochondria, reacts catalytically to form multiple ROS, including hydroxyl radicals, singlet oxygen, and the superoxide anion.\textsuperscript{10,11} These species, with half-lives between a nanosecond and a millisecond in solution, form DNA adducts, crosslinks, and strand-breaks but are difficult to observe due to their short lifetimes.\textsuperscript{10,12} A label-free sensor which converts chemical information immediately into a near-infrared signal would be a promising tool for studying these challenging bioanalytes.

6.2 MULTI-MODAL DETECTION AND ANALYTE FINGERPRINTING

Figure 6.1 outlines four reaction pathways which we measure via SWNT optical modulation. In the first reaction, the d(GT)\textsubscript{15} oligonucleotide-bound nanotube\textsuperscript{13} (DNA-SWNT) is exposed to a chemotherapeutic alkylating agent (melphalan shown) which reacts with the guanine nucleobase. This results in a uniform red-shift in the photoluminescence bands of both (6,5) and (7,5) nanotubes (Figure 6.2a). The second reaction shows direct adsorption of hydrogen peroxide with the nanotube which results in attenuation of both nanotubes’ emission and a slight concomitant energy shift (Figure 6.2b). Singlet oxygen, generated by exposing the nanotube complexes to Cu\textsuperscript{2+} and H\textsubscript{2}O\textsubscript{2}, causes a pronounced red-shift of the (6,5) nanotube emission, but no corresponding shift in the (7,5) band (Figure 6.2c). Finally, hydroxyl radicals, produced in the presence of SWNT by Fe\textsuperscript{2+} and H\textsubscript{2}O\textsubscript{2}, damage the DNA backbone and attenuate both nanotubes’ emission but preferentially affect the (7,5) emission, without energy shifts (Figure 6.2d). These spectral changes can be monitored transiently, elucidating the dynamic behavior of
each agent and the clear differences among them (Figure 6.3). The same trends are
evident upon varying genotoxin concentration (Figure 6.4).
Figure 6.1: Scheme of interactions on the DNA-SWNT complex: alkylating agent reaction with guanine, hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) adsorption on the nanotube sidewall, singlet oxygen (\textsuperscript{1}O\textsubscript{2}) reaction with DNA, and hydroxyl radical (\textbullet OH) damage to DNA.
Figure 6.2: (a) DNA-SWNT photoluminescence spectra before (blue) and after (green): introducing mechlorethamine (blue border), (b) hydrogen peroxide (magenta border), (c) singlet oxygen (orange border), and (d) hydroxyl radicals (green border).
Figure 6.3: (a) Transient responses of photoluminescence intensity (top) and energy (bottom) of the (6,5) nanotube (black) and (7,5) nanotube (red) upon introducing mechlorethamine, (b) hydrogen peroxide, (c) singlet oxygen, and (d) hydroxyl radicals. Responses were measured in Tris buffer.
Figure 6.4: Concentration dependence of SWNT response to genotoxins. Plots of (6,5) and (7,5) SWNT energy and intensity upon varying genotoxin concentrations in buffered 5 mg/L CoMoCAT SWNT solution. (a) Mechlorethamine response acquired 7 hours after addition. (b) Hydrogen peroxide response acquired 24 hours after addition. (c) Singlet oxygen response acquired 1 hour after addition of hydrogen peroxide. (d) Hydroxyl radical response acquired 10.5 hours after addition of hydrogen peroxide. Data was acquired approximately when reagent reached steady-state, with the exception of singlet oxygen due to difficulty of deconvolution of overlapping (6,5) and (7,5) bands over long reaction times.
Exposing DNA-SWNT to several analytes simultaneously achieves signal multiplexing. Concomitant generation of hydroxyl radicals and singlet oxygen elicits a precipitous initial intensity drop and pronounced (6,5) band intensity shifts, corresponding to the production of both analytes (Figure 6.5). Subsequent analysis described below confirms multiplexed detection.

Principal components analysis\(^{14}\) (PCA) validates analyte fingerprinting. The subspace determined by the first two principal component scores of all transient and concentration-dependent (6,5) and (7,5) response data exhibits segregation by analyte (Figure 6.6). The analysis was conducted on all available sensor data assembled into a matrix of four columns, one for each of four \(x_i\) measurements: (6,5) SWNT photoluminescence band energy and intensity, and (7,5) band energy and intensity. Early data points (zero concentration and zero time) were removed to clarify the plots, although their inclusion had negligible effect on the PCA. The first and second principal components were PC1 = 0.9572X1 + 0.1073X2 + 0.2274X3 + 0.1433X4 and PC2 = -0.2729X1 + 0.4367X2 + 0.5071X3 + 0.6911X4.\(^{15}\)

This confirms analyte identification based on the four signal transduction modes. Analysis of simultaneous reagent detection (from Figure 6.5) verifies multiplexed detection, as early time points cluster in the hydroxyl radical and \(\text{H}_2\text{O}_2\) zones, while later points situate in the singlet oxygen region. We investigated the nature of all analyte responses as described below.
Figure 6.5: Multiplexed detection of singlet oxygen and hydroxyl radicals. Plots of (6,5) and (7,5) intensity and energy upon inducing both singlet oxygen and hydroxyl radical production in the presence of 5 mg/L CoMoCAT DNA-SWNT show characteristics of both agents. With increasing FeSO₄/decreasing CuCl₂ concentration, the initial intensity drop of the (7,5) nanotube is more pronounced and remains for a longer duration. Concomitantly, the rate of (6,5) energy shift decreases. (a) Intensity (top) and energy (bottom) with 0.02 mM FeSO₄, 0.08 mM CuCl₂ and 10 mM H₂O₂. (b) 0.04 mM FeSO₄, 0.06 mM CuCl₂ and 10 mM H₂O₂. (c) Plots showing concentration dependence of spectral changes. Data was acquired 60 minutes after introduction of reagents. FeSO₄ and CuCl₂ concentrations were varied in compensatory fashion (with the exception of the 0,0 data point), while H₂O₂ concentration remained at 10 mM.
6.3 CHEMOTHERAPEUTIC DRUG SENSING

Chemotherapeutic alkylating agents are detected immediately upon exposure to the DNA-SWNT complex. Emission red shifts of up to 6 meV and concomitant attenuation were observed via n-IR spectrofluorimetry (Figures 6.7 and 6.8). The red shifting rates caused by nitrogen mustards melphalan and mechlorethamine are similar (first order $k_{\text{obs}} = 0.014 \text{ min}^{-1}$ and $0.012 \text{ min}^{-1}$ respectively), while cisplatin promotes three-fold lower shift rate ($k_{\text{obs}} = 0.0047 \text{ min}^{-1}$). The rate of drug alkylation to an unbound test oligonucleotide containing one guanine nucleobase, analyzed by polyacrylamide gel electrophoresis, is similar to the rate of drug-induced DNA-SWNT shift ($k_{\text{obs}} = 0.0083 \text{ min}^{-1}$ for mechlorethamine and $k_{\text{obs}} = 0.0058 \text{ min}^{-1}$ for cisplatin; direct comparison to
d(GT)$_{15}$ is provided in Figure 6.9). The DNA-SWNT response of melphalan is slower than the unbound DNA alkylation ($k_{obs} = 0.028 \text{ min}^{-1}$), which we attribute to the steric bulk of this larger molecule as it interacts with the nanotube-bound DNA. The rates of SWNT emission shifting are highly sequence-dependent, proportional to the abundance of guanine in the bound oligonucleotide sequence (Figure 6.10), which is consistent with the known preference of melphalan for alkylating purine nucleobases.$^5$ We conclude that the DNA-SWNT complex emission responds to the alkylation of the bound oligonucleotide via a solvatochromic shift mechanism$^2$ due to the introduction of the drug adduct into the immediate vicinity of the nanotube, resulting in conformational changes of the encapsulating DNA.

![Graph showing wavelength vs relative intensity](image)

**Figure 6.7:** Detection of chemotherapeutic drugs using HiPco SWNT. The DNA-SWNT complex prepared using HiPco SWNT behaved in a qualitatively similar manner to the complex fabricated with CoMoCAT SWNT. Red-shifting of both (6,5) and (7,5) SWNT species is observed upon introduction of melphalan (red) compared to the control spectrum (blue).
Figure 6.8: Mechanistic studies of SWNT-genotoxin reactions. (a) Photoluminescence shift of (6,5) nanotube (blue curve) upon DNA-SWNT exposure to melphalan. Kinetics trace of unreacted oligonucleotide band intensity (red curve) from 20% PAGE (inset) upon melphalan-induced alkylation of an unbound test sequence. (b) Mechlorethamine-induced response. (c) Cisplatin response.
Figure 6.9: Comparison between damage to free d(GT)$_{15}$ DNA and DNA-SWNT fluorescence red-shift from alkylating agent activity. (a) PAGE kinetic study of 0.9 mM melphalan reaction on the d(GT)$_{15}$ oligonucleotide sequence. (b) Normalized intensity of the remaining d(GT)$_{15}$ band from PAGE (black squares) plotted against the red-shift of d(GT)$_{15}$ encapsulated SWNT exposed to melphalan (red diamonds) under the same conditions. The d(GT)$_{15}$ oligonucleotide did not produce distinct bands in the gel due to multiple guanine damage sites, prompting the use of a test sequence containing only one guanine base (Figure 6.8). The test sequence exhibits the same kinetics as shown above upon exposure to melphalan.
Figure 6.10: Sequence dependence of alkylating agent detection by DNA-SWNT. Rate of (6,5) nanotube photoluminescence red-shift upon melphalan exposure to nanotubes encapsulated by sequences of varying G/T ratios. Nanotube-DNA complexes with sequences containing a higher fraction of guanine display higher reactivity to alkylating drugs, consistent with the observed behavior of nitrogen mustard agents\textsuperscript{5}.

6.4 DETECTION OF REACTIVE OXYGEN SPECIES

Hydrogen peroxide is detected by the DNA-SWNT complex via attenuation of both (6,5) and (7,5) fluorescence bands to similar extents with slight shifting of peak wavelengths (Figure 6.2). This behavior is attributed to reversible charge-transfer quenching upon H$_2$O$_2$-SWNT contact, caused by peroxide’s high reduction potential.\textsuperscript{16} While (6,5) and (7,5) nanotube signals show similar attenuation in this study, small bandgap nanotubes attenuate to a greater extent, in agreement with absorption spectroscopy studies\textsuperscript{17} (Figure 6.11). This provides additional basis for fingerprinting hydrogen peroxide for studies involving small-bandgap SWNT species.
Figure 6.11: Hydrogen peroxide photoluminescence quenching of DNA-SWNT. The hydrogen peroxide response of DNA-SWNT using HiPco nanotubes excited at 633 nm demonstrates SWNT species dependence. (a) The spectrum taken after exposure to 10 mM H₂O₂ for 24 hours (red curve) shows greater attenuation of small bandgap nanotubes (emitting at longer wavelengths) compared to the control spectrum (blue curve). Large bandgap nanotubes (such as the (7,5) species) exhibit less attenuation. (b) Transient attenuation of three SWNT species showing higher rates for smaller bandgap semiconducting species. The reaction was conducted in 20 mM Tris at a pH of 7.3 with 0.1 M NaCl.

Singlet oxygen formed in the direct vicinity of the DNA-nanotube complex induces a pronounced red-shift in the (6,5) nanotube emission with virtually no (7,5) nanotube shift (Figure 6.2), as confirmed by the 3D photoluminescence profile (Figure 6.12). Shifts of over 60 meV (50 nm) have been observed, as well as small red and blue shifts of other SWNT species along with relative attenuation of large bandgap species (Figure...
6.13). Similar trends are seen in absorption spectra (Figure 6.14). The effect of singlet oxygen on the DNA-SWNT signal is sequence-dependent, as various strands used to encapsulate the nanotube in place of d(GT)$_{15}$ promote shift rates roughly proportional to the abundance of purine nucleobases in the sequence (Figure 6.15); purines are the most easily oxidized nucleobases.\textsuperscript{18} The rate measured among several metal ion catalysts (Figure 6.15 inset) is greater for ions that demonstrate higher binding affinity to nucleobases\textsuperscript{19}, suggesting that singlet oxygen causes the red shift when produced in the vicinity of the nucleobases. By preventing singlet oxygen generation in the nanotube’s immediate vicinity, via chelating all available Cu$^{2+}$ ions with EDTA\textsuperscript{20}, the wavelength shift is completely inhibited and signal attenuation is reduced (Figure 6.16). Exposure to the singlet oxygen scavenger, sodium azide, significantly reduces the magnitude of the (6,5) band shift (Figure 6.17); however, its introduction after shifting does not cause a reversal. These experiments suggest that this unique SWNT response is due to a singlet oxygen-induced DNA adduct resulting from nucleobase oxidation, such as 8-oxo-deoxyguanosine\textsuperscript{21}. The oxidized nucleobase increases the polarity of the nanotube’s microenvironment, causing a solvatochromic red-shift. Response variations between nanotubes result from SWNT structural and electronic differences which cause DNA-nanotube interactions to vary across species, resulting in diverse responses to DNA adduct formation. Identification of the dominant adduct formed in this case will be the focus of future work.
Figure 6.12: 3D photoluminescence profiles of DNA-SWNT before (top) and after (bottom) inducing singlet oxygen generation. (Nanotubes synthesized by CoMoCAT method.)
Figure 6.13: Singlet oxygen reaction on DNA-SWNT. (Nanotubes produced by HiPco method.) A 3D photoluminescence profile of 5 mg/L DNA-SWNT using HiPco nanotubes before (top) and 24 hours after (bottom) exposure to CuCl₂ and hydrogen peroxide in 20 mM Tris buffer with a pH of 7.3 and 0.1 M NaCl. The (6,5) nanotube undergoes a large red shift (circled in bottom trace). Relative intensity changes of nanotube species occur, and total intensity of all peaks fall, demonstrating similar behavior to CoMoCAT prepared SWNT (Figure 6.12). Plot intensities were normalized independently. The CoMoCAT SWNT preparation, whose (6,5) and (7,5) relative abundances are approximately 2:1, and whose (6,5) concentration is near 40% of the total SWNT content, was chosen for most of the sensing work presented here, over the HiPco preparation, whose approximate (6,5) and (7,5) fractional intensities are 3.7% and 4.9% respectively of the total photoluminescence in HiPco SWNT.²²,²³
Figure 6.14: Singlet oxygen shifts SWNT absorption bands. Absorption spectra of 5 mg/L d(GT)$_{15}$ encapsulated CoMoCAT SWNT before (blue) and 24 hours after (red) inducing singlet oxygen production. The (6,5) band exhibits a red-shift while the (7,5) band shows little change in wavelength. The E$_{22}$ bands exhibit shifting to a lesser degree than E$_{11}$ bands. The samples were buffered in 20 mM Tris at a pH of 7.3 with 0.1 M NaCl.

Figure 6.15: Rate of singlet oxygen-induced (6,5) band red-shifting of SWNT encapsulated by oligonucleotide sequences of increasing purinic character (from left to right; see Methods for Seq 1 – 67% purines). Inset: Rate of (6,5) band red shift induced by singlet oxygen generated by H$_2$O$_2$ and cations with increasing nucleobase-binding capability (from left to right).
Figure 6.16: Chelation of Cu\(^{2+}\) with EDTA prevents all (6,5) peak shifting (red) upon introduction of H\(_2\)O\(_2\). Chelating either Fe\(^{2+}\) or Cu\(^{2+}\) reduces (6,5) signal attenuation (blue) upon introducing H\(_2\)O\(_2\).

Figure 6.17: Sodium azide diminishes (6,5) nanotube energy shift triggered by Cu\(^{2+}/\)H\(_2\)O\(_2\).

Generation of the hydroxyl radical by the Fenton reaction\(^{10}\) is detected by the DNA-SWNT complex via attenuation of the nanotube’s photoluminescence. The Fe\(^{2+}\) ion catalyzes hydroxyl radical formation in the presence of H\(_2\)O\(_2\), which, in the vicinity of DNA-SWNT, greatly attenuates both (6,5) and (7,5) fluorescence bands without shifting.
the peak wavelengths and generally attenuates the (7,5) nanotube emission to a greater extent (Figure 6.2). Chelation of all available Fe²⁺ with EDTA reduces, but does not eliminate, signal attenuation, suggesting that damage can be detected without close association of Fe²⁺ to the nucleobases (Figure 6.16). Mannitol, a hydroxyl radical quencher²¹, prevents signal attenuation when added before initiating the reaction (Figure 6.18). Hydroxyl radical interaction with DNA-SWNT exhibits highly disproportional attenuation of small-bandgap species emission (Figure 6.19). We thus conclude that the DNA-SWNT complex detects the hydroxyl radical by induced DNA damage. The resulting DNA adduct includes a species which induces photoluminescence attenuation by a charge-transfer mechanism.

![Figure 6.18](image)

**Figure 6.18:** Mannitol reduces Fe²⁺/H₂O₂ induced signal attenuation of DNA-SWNT.
Figure 6.19: Hydroxyl radical detection by DNA-SWNT. DNA-SWNT detects hydroxyl radicals by species-specific quenching. The photoluminescence quenching of smaller bandgap SWNT is highly disproportionate upon exposure of Fenton reagents to DNA-encapsulated HiPco-SWNT (633 nm excitation).

6.5 SINGLE-MOLECULE DETECTION OF HYDROGEN PEROXIDE

Stepwise n-IR photoluminescence quenching of surface-tethered DNA-SWNT complexes demonstrate single-molecule detection of hydrogen peroxide. Recent work has shown that analyte-SWNT interactions can be studied at the single-molecule level via immobilization of surfactant-suspended nanotubes in agarose. We encapsulated nanotubes with a 1:4 ratio of biotinylated to non-biotinylated d(GT)15, allowing Neutravidin-specific binding of DNA-SWNT to a BSA-biotin treated surface (Figure 6.20). Immobilized DNA-SWNT complexes were imaged via their n-IR photoluminescence signal upon laser excitation (Figure 6.21). Time traces of SWNT quenching (Figure 6.22) were obtained by measuring the intensity of 4-pixel spots in movies recorded at 1 frame/s, resulting in multiple traces which exhibit single-step attenuation upon perfusion of hydrogen peroxide. The traces yield a narrow histogram of
normalized quantized intensity changes after regression with a stochastic step-fitting algorithm\textsuperscript{25}, confirming the discrete nature of the interaction (Figure 6.23). The average normalized step height of 0.05-0.1 is consistent with a 90 nm exciton excursion range\textsuperscript{24} and spot size of 900 nm, confirming a quantized single-step magnitude of 0.1. These measurements demonstrate single-molecule detection of hydrogen peroxide, providing promise for new classes of biosensors with this level of sensitivity.

\textbf{Figure 6.20:} Schematic of biotinylated DNA-SWNT binding to a glass surface via BSA-biotin and Neutravidin.

\textbf{Figure 6.21:} Single near-infrared movie frame showing photoluminescence from several DNA-SWNT complexes (scale bar measures 10 µm).
Figure 6.22: Fitted traces from movie showing single-step SWNT emission quenching upon perfusion of hydrogen peroxide.

Figure 6.23: Histogram of fitted step sizes from five traces taken from one n-IR movie.
6.6 CELLULAR DETECTION AND IDENTIFICATION OF ANALYTES

Nucleic acid encapsulated SWNT enter cells via endocytosis without exhibiting cytotoxic effects at doses of at least 5 mg/L\textsuperscript{26} while maintaining their photoluminescence properties,\textsuperscript{27,28} which can be perturbed in situ\textsuperscript{3}. Colocalization images of nanotube fluorescence in murine 3T3 cells with a lysosomal stain show partial overlap, suggesting DNA-SWNT presence in both lysosomes and the cytoplasm (Figure 6.24). Perfused chemotherapeutic drugs and ROS induce SWNT spectral changes in real time within live cells, allowing detection in situ using this multi-modal technique. The photoluminescence intensity changes of DNA-SWNT, upon interaction with genotoxins, can be spatially resolved within single cells, as shown in images before and after inducing hydroxyl radical formation (Figure 6.25).

Figure 6.24: Real-time multiplexed detection of genotoxins in live mammalian cells. (Left) Fluorescence of lysosomal stain Lysotracker in 3T3 cells. (Right) DNA-SWNT photoluminescence (green) showing partial colocalization with Lysotracker emission. Scale bars measure 20 µm.
Four genotoxins are demonstrably detected and identified in live 3T3 cells spectroscopically. Real-time measurements reveal that the alkylating agent mechlorethamine induces a detectable attenuation and peak shift of both (6,5) and (7,5) nanotube bands following perfusion of the drug into cell media, similar to in vitro behavior (Figure 6.26). Perfusion with hydrogen peroxide alone induces a temporary attenuation of both nanotube bands, with minimal shifting, that reverses within 5-10 minutes (Figure 6.26b). The reversibility is expected due to cellular enzymes which decompose hydrogen peroxide. Singlet oxygen (Figure 6.26c) and hydroxyl radicals (Figure 6.26d), generated in vivo by perfusion of their respective catalytic reagents, producing trends generally consistent with the DNA-SWNT response in vitro, although intensities exhibit some deviation. Such events are expected during detection in single cells, as cell movement can cause intensity fluctuations. Additionally, endosomal
localization and the DNA coating may lower the detection limit due to aggregation caused by endosome fusion and protein binding to DNA-SWNT. Analysis via PCA reveals that cellular responses uniquely fingerprint each genotoxin. A principal components plot (Figure 6.27) shows analyte data separated into distinct regions, consistent with in vitro data (Figure 6.6).
Figure 6.26: Analyte responses detected within 3T3 cells. (a) Photoluminescence intensity (top graph) and energy (bottom graph) of the (6,5) nanotube (black trace) and (7,5) nanotube (red trace) after introducing mechlorelthamine (blue borders), (b) hydrogen peroxide (magenta borders), (c) singlet oxygen (orange borders), and (d) hydroxyl radicals (green borders). Arrows denote time of agent addition.
Figure 6.27: Principal components analysis of analyte responses taken within 3T3 cells showing segregation of data into discrete regions. Area-minimized ovals encompass regions defined by all available data for each analyte. Arrows denote direction of increasing time.

In conclusion, we report that single-walled carbon nanotubes can act as a unique multimodal sensor for separate reaction pathways and biologically relevant analytes. The findings introduce new spectral phenomena heretofore unknown in carbon nanotubes. Moreover, the work demonstrates the first multiplexed optical detection from a nanoscale sensor and provides the first tool to identify and measure both alkylating and ROS activity in real time within living cells. The specificity and versatility of detection portend significant and numerous diagnostic and metrologic applications for this approach.
6.7 EXPERIMENTAL METHODS

6.7.1 SWNT preparation

Raw HiPco SWNT (Rice University) were suspended via bath ultrasonication for 1 hour in a 1:1 mass ratio with the d(GT)$_{15}$ oligonucleotide. The resulting solution was centrifuged at 16,300 g for 90 minutes and the pellet was discarded. Raw CoMoCAT SWNT (Southwest Nanotechnologies) were suspended via probe-tip ultrasonication at 10 W for 10 minutes in a 4:1 ratio with d(GT)$_{15}$ in 0.1 M NaCl cooled by an ice bath. The resulting GT-CoMoCAT solution was centrifuged at 16,300 g for 90 minutes and the pellet was discarded.

6.7.2 Spectroscopy and microscopy

Near-infrared photoluminescence spectra were acquired using 785 nm excitation and an Acton SP-150 spectrograph coupled to a Princeton Instruments OMA V InGaAs detector or with a Kaiser Holospec f/1.8 Imaging Spectrograph (Kaiser Optical). Absorption measurements were taken with a Shimadzu UV-3101 PC UV-VIS-NIR Scanning Spectrophotometer. Photoluminescence 3D profiles were acquired with an in-house built spectrofluorometer incorporating a xenon arc lamp, Kratos GM-252 monochromators, and E1-L Germanium Detector (Edinburgh Instruments). Single-molecule and cell microscopy studies used a Carl Zeiss Axiovert 200 fluorescence microscope coupled to a Princeton Instruments 2D-OMA V InGaAs camera with a 256 x 320 pixel array and Acton spectrograph. Visible fluorescence images were acquired with an AxioCam MRm CCD camera. Spectra were processed by fitting to a Gaussian lineshape to determine peak center wavelength.
6.7.3 Chemotherapeutic agent kinetics

To elucidate drug-induced shifting, a solution of 5 mg/L of GT-CoMoCAT SWNT in 100 mM Tris buffer (pH 7.4) was exposed to 20 mM mechlorethamine, and spectra were acquired at room temperature. For kinetic measurements, 5 mg/L of GT-HiPco in 100 mM Tris buffer (pH 7.4) was exposed to the alkylating agent melphalan, mechlorethamine, or cisplatin at a concentration of 0.5 mM. Photoluminescence spectra were taken over 400-600 minutes at 37 °C. Unbound oligonucleotide kinetics were measured via polyacrylamide gel electrophoresis (PAGE) on the test sequence: 5'-TTT TTG TTT T-3’. The sequence was labelled with 32P at the 5’ end and exposed to 0.5 mM of alkylating agent in 100 mM Tris (pH 7.5) at 37 °C. Aliquots were removed at each time point and held at -80 °C before 20% PAGE.

6.7.4 Reactive oxygen species

Transient photoluminescence measurements were conducted by exposing GT-CoMoCAT SWNT in 20 mM Tris (pH 7.3) and 0.1 M NaCl (henceforth “buffer”) to 10 mM H2O2 in the case of hydrogen peroxide experiments. Singlet oxygen was induced with 0.1 mM CuCl2 and 10 mM H2O2; hydroxyl radicals with 0.05 mM FeSO4 and 10 mM H2O2. Cations were added to DNA-SWNT in buffer one hour prior to the addition of H2O2 to rule out cation-induced effects31. Spectra were acquired over 10-400 minutes. Photoluminescence 3D profiles were taken using 5 mg/L GT-CoMoCAT SWNT in buffer with CuCl2. The second profile was taken 24 hours after exposure to 3.6 mM H2O2. All ROS experiments were conducted at room temperature.
6.7.5 ROS measurements after cation chelation

Solutions of 5 mg/L of GT-CoMoCAT SWNT in buffer plus 0.1 mM CuCl₂ or 0.1 mM FeSO₄ and 2 mM EDTA were made prior to reacting with 1 mM H₂O₂. Photoluminescence measurements were taken 24 hours later.

6.7.6 ROS measurements using different DNA sequences/cations

Solutions of 5 mg/L HiPco SWNT encapsulated by five different DNA sequences were made using the bath ultrasonication method described above. Sequences were d(T)₃₀, d(GGTT)₇TT, d(GAT)₁₀, d(GGGGT)₆ and an oligonucleotide denoted Seq 1 containing the 27-nucleotide sequence 5’-ACC TGG GGG AGT ATT GCG GAG GAA GTT -3’ (purinic content = 67%). All other sequences were 30 nucleotides long. Photoluminescence of each sequence was measured upon reaction with 0.1 mM CuCl₂ and 3.6 mM H₂O₂ in buffer. Photoluminescence of GT-HiPco was measured 24 hours after reaction of 0.1 mM of various metallic cations with 3.6 mM H₂O₂ after 24 hours to determine cation dependence on shift.

6.7.7 Cell culture

Murine NIH/3T3 cells were cultured with HEPES-buffered Dulbecco’s Minimal Essential Media (Sigma) supplemented with 10% fetal bovine serum (Biomedia, Foster City, CA). The cells were incubated with 2 mg/L of GT-CoMoCAT SWNT for 6-8 hours before trypsin digestion and transferring to a separate container. Cells were plated on glass-bottom petri dishes (MatTek) for microscopy.
6.7.8 Lysosomal imaging and photoluminescence/visible overlays

Lysosomal dye colocalization images were taken two hours after exposing cells containing GT-CoMoCAT SWNT to 70 nM of LysoTracker Red (Invitrogen). Cells were alternately exposed to white light excitation passed through a rhodamine dye filter cube for dye excitation and a 785 nm laser for SWNT imaging. Nanotube photoluminescence overlays on visible cell images were acquired by exposing the cells alternately to halogen epi-illumination and 785 nm excitation with detection by 2D InGaAs camera.

6.7.9 Live cell drug/ROS perfusion

Cells adhered to glass-bottom Petri dishes were imaged inside a micro-incubation platform (Model DH-40i, Warner Instruments, Inc). Alkylating drugs and other reagents were perfused via syringe. Cells were bathed in media with FBS during chemotherapeutics experiments. Mechlorethamine dissolved in DMEM media with FBS was perfused to reach a final concentration of 50 mM. Media was exchanged for saline solution just before ROS experiments to prevent cation precipitation and hydrogen peroxide degradation. The salts CuCl₂ or FeSO₄ were perfused approximately 30 minutes before measurements, at 1 mM and 0.1 mM, respectively, while 30 mM H₂O₂ was perfused during data acquisition. Laser power was limited to 1.1 mW.
6.7.10 Single-molecule studies

Nanotubes were encapsulated with DNA via probe-tip ultrasonication in a 4:1 DNA:HiPco SWNT ratio for 2 min. A ratio of 1:4 biotinylated:non-biotinylated d(GT)$_{15}$ DNA was used to produce complexes with multiple biotinylated oligonucleotides per SWNT. Solutions were centrifuged at 16,000 g for 90 minutes and the pellet was discarded. A sample chamber for single-molecule experiments was created as described$^{32}$. The surface was successively treated with 1 mg/ml biotinylated-BSA in T100 (10 mM Tris [pH 8.0] and 0.1 M NaCl) and 0.2 mg/ml Neutravidin in T100. Biotinylated DNA-SWNT (concentration approximately 1 mg/L) in T100 was added to the sample chamber and incubated for at least 30 minutes before imaging. Channels were flushed with deionized water before imaging. Near-IR movies were captured at 1 frame/s using 633 nm excitation. An aliquot of 10 µM H$_2$O$_2$ was dropped on the inlet hole of the slide and allowed to diffuse into the sample chamber during data acquisition.

6.7.11 Concentration-dependent genotoxin responses

Buffered solutions of 5 mg/L GT-CoMoCAT SWNT were prepared to expose the SWNT complexes to six different concentrations of each genotoxin: mechlorethamine, H$_2$O$_2$, singlet oxygen, and hydroxyl radicals. The latter two were prepared by first adding several concentrations of CuCl$_2$ for singlet oxygen or FeSO$_4$ for hydroxyl radicals one hour before initiating the reactions with 10 mM H$_2$O$_2$. Spectra were acquired at a single time point for each genotoxin.
6.7.12 Multiplexed detection experiments

Solutions of 5 mg/L of GT-CoMoCAT SWNT in buffer were exposed to mixtures of CuCl₂ and FeSO₄ in several ratios. Cations were introduced to the solutions one hour before starting the reactions with 10 mM H₂O₂. Multiple near-infrared spectra were recorded on samples over a five-hour period for transient spectra, or after 1 hour of reaction time for concentration-dependent studies.

6.7.13 Alkylating agent detection by DNA-SWNT upon varying DNA sequence

Solutions of 5 mg/L HiPco SWNT were encapsulated by d(T)₃₀, d(GT)₁₅, and d(GGGGT)₆ via bath ultrasonication for 1 hour in a 1:1 SWNT:DNA mass ratio. The resulting solution was centrifuged at 16,300 g for 90 minutes and the pellet was discarded. The near-infrared photoluminescence of each DNA-SWNT complex was measured upon addition of 0.9 mM melphalan in 100 mM Tris buffer (pH 7.4).

6.8 LIST OF REFERENCES


15. The first and second principal components represent 89% of the variance.


CHAPTER 7: DETECTION AND IDENTIFICATION OF NITRO COMPOUNDS

7.1 INTRODUCTION AND BACKGROUND

As outlined in previous chapters, semiconducting SWNT emit bandgap photoluminescence (PL) in the near-infrared (NIR) region of the electromagnetic spectrum\textsuperscript{1,2} which is responsive to physical and chemical changes to the nanotube’s environment.\textsuperscript{3} It is made selectively responsive by specific polymer coatings adhered to the walls of the SWNT.\textsuperscript{4,5}

The mechanisms of signal transduction include charge transfer interactions\textsuperscript{6}, which alter the PL intensity, and solvatochromic shifts\textsuperscript{7}, which change the wavelength of the PL\textsuperscript{8}. These two responses constitute separate modes of detection from the same nanotube emission band, allowing differentiable, analyte-dependent responses. Moreover, the existence of multiple semiconducting SWNT species with different bandgaps and photoluminescence emission wavelengths, as well as varying responsiveness to electronic structure perturbations, results in analyte-specific spectral signatures, which allows fingerprinting of analytes, as demonstrated in Chapter 6.\textsuperscript{4}

Stochastic sensing of single-molecule adsorption events on SWNT was also demonstrated.\textsuperscript{9} We have previously demonstrated stepwise quenching of nanotube photoluminescence by single molecules of hydrogen peroxide upon adsorption to the nanotube sidewall\textsuperscript{4,10}.

Detection of explosives and other nitro group-containing compounds is an outstanding contemporary research problem.\textsuperscript{11} Conventional methods for sensitive detection of explosives containing nitro groups include ion mobility spectroscopy and mass spectrometry.\textsuperscript{12} Nitroaromatic pesticides such as 2,4-dinitrophenol have been
measured via HPLC-UV and HPLC-MS. The above methods largely do not operate in real-time, and the equipment is not portable for detection in the field. Detection schemes which employ these capabilities are currently in discovery and, to some extent, in employment stages. Fluorescent polymers have been recently employed to detect trinitrotoluene (TNT) and similar nitroaromatics via binding and quenching interactions. Researchers recently used phage display techniques of peptide selection to discover an oligopeptide which binds to trinitrotoluene (TNT) and dinitrotoluene (DNT) with detection studies conducted via quartz crystal microbalance. Electronic detection of TNT via carbon nanotubes was recently demonstrated using a SWNT-FET device decorated with a TNT-binding peptide identified by phage display.

This work looks at a series of polymers and biopolymers used to encapsulate SWNT for nitro compound detection, including polyvinyl alcohol (PVA) and the d(GT) DNA oligonucleotide, as well as peptides, which, as we have discovered, selectively detect and transduce the binding of nitro group-containing compounds. The bombolitin family of oligopeptides contains five species with 17-residue sequences derived from bumblebee venom. The sequences of the variants used here are bombolitin I: IKITTMLAKLGKVLHV, bombolitin II: SKITDILALGKVLHV, bombolitin III: IKIMDLALGKVLHV, and bombolitin IV: INIKDILAKLVLGTV. Their conformations are largely disordered in aqueous solution; in the presence of bilipid membranes, they take on a more ordered, alpha helical structure according to circular dichroism studies. At high concentrations, above 2.5 mM for bombolitin III for instance, and higher for other sequences, the peptides form aggregates with an anti-parallel alpha-helical conformation.
This effort investigates two mechanisms for nitro-containing species to be selectively recognized by biopolymer-encapsulated SWNT. Trinitrotoluene (TNT) is detected via redox-induced bleaching, with selectivity determined by the adsorbed oligonucleotide, while other nitro compounds, including RDX, picric acid, and two pesticides, are recognized and identified by a class of helix-coil peptides which undergo a conformational change upon binding to the analyte compound, resulting in a solvatochromic shift of the nanotube photoluminescence. The work constitutes an optical sensor for real-time explosives detection, with the abilities of analyte fingerprinting and single-molecule sensitivity. We also demonstrate the first carbon nanotube-based optical sensor which detects a peptide secondary structure change.

7.2 WRAPPING-MEDIATED RESPONSES TO REDOX-ACTIVE COMPOUNDS

We find that altering the polymer or peptide wrapping around a single walled carbon nanotube modulates analyte responsiveness. We first compare the spectral fluorescent intensity and wavelength changes of several polymer and peptide wrapped SWNT upon exposure to an array of various analytes.

Measurements conducted on nanotubes solubilized with several polymers highlight the role of the polymer in permitting detection specificity. First, we note that solvatochromic shifts from a particular analyte are rare. The (7,5) nanotube, solubilized by polyvinyl alcohol (PVA-SWNT), undergoes intensity attenuation upon exposure to certain analytes (ascorbic acid, NADH, dopamine, L-thyroxine, melatonin, and serotonin) (Figure 7.1, top). The apparent wavelength shifting of SWNT exposed to the two
analytes which provoke the greatest responses is in fact an artifact caused by low signal after attenuation.

Upon introduction of the analytes to nanotubes encapsulated by the d(GT)$_{15}$ oligonucleotide, many of the same compounds cause intensity changes as with PVA-SWNT, with the addition of tyramine, riboflavin, tryptophan, and trinitrotoluene (TNT). Some analytes induce a signal increase, with the exceptions of riboflavin and TNT, which induce the sole quenching effects on this nanotube complex. The modulation of the intensity response relative to that of PVA-SWNT is due to a doping effect caused by the DNA wrapping, which likely p-dopes the nanotube relative to the PVP wrapping.
Figure 7.1: Analyte screening results against PVA-solubilized SWNT (top) and d(GT)15-encapsulated SWNT (bottom). Intensity changes of the (7,5) nanotube species relative to the control ($I/I_0$), as well as the wavelength shift in nanometers relative to the control ($E-E_0$) are shown for each analyte. (All error bars indicate one standard deviation.)
The attenuation of photoluminescence intensity in response to redox-active compounds have been attributed to electron transfer which shift the Fermi level of the nanotube, resulting in spectral bleaching.\textsuperscript{6,20,21} This mechanism explains the PVA and d(GT\textsubscript{15} DNA encapsulated SWNT analyte responses. Figure 7.2 plots the relative position of the Fermi level for each carbon nanotube species, obtained from Doorn, et, al.\textsuperscript{20} Reduction potentials of several analytes vs. the normal hydrogen electrode (NHE) were obtained from the literature and appear as horizontal lines. Attenuating analytes dopamine, riboflavin, ascorbic acid, and NADH exhibit higher reduction potentials than the Fermi levels of several nanotube species, signifying the possibility of charge transfer to the nanotube. The non-bleaching analyte salicylic acid exhibits a reduction potential well below the SWNT Fermi level. The Fermi level has not been adjusted for the type of encapsulating polymer, due to the uncertainty each polymer’s effect on the electronic structure of the nanotube.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure7.2.png}
\caption{Carbon nanotube Fermi levels in V plotted against SWNT E\textsubscript{11} transition energies. The reduction potentials of several analytes are shown. The analytes’ relatively high reduction potentials as compared to the SWNT Fermi levels suggest charge transfer from the nanotube to the analytes will occur. The one exception is salicylic acid, whose reduction potential is much higher.}
\end{figure}
Nanotubes encapsulated by the d(GT)$_{15}$ oligonucleotide respond selectively to TNT, which elicits a quenching response (Figure 7.3). Few other such compounds quench with the exception of 2,4-dinitrotoluene and 2-nitrophenol, to lesser degrees. There are also few wavelength shifting responses. The reduction potentials of three nitro compound-containing analytes are shown as horizontal lines in Figure 7.4 to compare to the SWNT Fermi level. The relative reduction potentials follow the analyte responses in Figure 7.3, as TNT and 2,4-dinitrotoluene are positioned to withdraw electron density from several SWNT species, while 2-nitroanaline is not.

![Figure 7.3: d(GT)$_{15}$-encapsulated SWNT responses to nitro-containing compounds.](image)

Intensity changes of the (11,3) nanotube species relative to the control (I/I$_0$), as well as the wavelength shift in nanometers relative to the control spectrum (E-E$_0$) are shown for each analyte.
7.3 A SOLVATOCHROMIC CHAPERONE SENSOR FOR NITRO COMPOUNDS

Nanotubes solubilized by the peptide bombolitin II experience wavelength shifts and only slight concomitant intensity variation in response to four compounds containing nitro groups: RDX, picric acid, 2,4-dinitrophenol, and 4-nitro-3(trifluoromethyl)phenol (TFM) (Figure 7.5). Redox-active analytes induce intensity reductions due to bleaching interactions explained above. Some compounds which induce precipitous attenuation in the SWNT PL intensity also induce apparent wavelength shifts which are spurious or exaggerated for reasons discussed earlier.
Figure 7.5: Bombolitin II-solubilized SWNT exhibits wavelength shifts and minimal intensity changes upon exposure to certain nitro compounds (blue arrows).

Further investigation of nitro group-containing compounds with bombolitin II-SWNT finds that 6 of 13 such compounds analyzed exhibit significant wavelength shifts, suggesting specificity beyond the simple presence of nitro groups, nitroaryls, or both phenyl and nitro groups (Figure 7.6). We also find that different \((n,m)\) nanotube species exhibit different detection signatures, where the intensity and wavelength changes vary across SWNT species. This variation is demonstrated here for the (7,5) and (11,3) species, which possess different diameters (0.829 nm vs 1.014 nm), chiral angles (24.5°...
vs 11.74°), and optical bandgaps (1.211 eV vs 1.036 eV). We have previously demonstrated the ability to identify analytes by exploiting the different signatures given by two SWNT species. Principal components analysis (PCA) performed on the detection data, from eight different SWNT (n,m) species, confirms unique signatures of the 6 analytes, denoted by their segregation into separate regions of the plot (Figure 7.7), allowing identification of the analytes by their responses. The analysis was conducted by compiling all eight nanotubes’ intensity change and wavelength shifting data for each analyte. The first three principal component scores, which account for a total of 99.5 % of the total data variance, are shown. Table 7.1 contains the loadings for the first three principal components listed by input variable.

Although all SWNT responses result from ring-structured compounds containing nitro groups, no recognizable structural component or pattern is present in the analytes which differentiates responses from non-responses (Figure 7.8). Although bombolitin II is a relatively short peptide, it is still difficult to predict binding events of such species, which accounts for the need for high-throughput selection methods such as phage display.22
Figure 7.6: Bombolitin II-solubilized nanotube responses to nitro compound responses. Intensity and wavelength responses of the (7,5) nanotube (top) and the responses of the (11,3) nanotube (bottom) differ. (Error bars indicate one standard deviation.)
Figure 7.7: Principal components analysis plot using the intensity and wavelength spectral data from bombolitin II-solubilized SWNT responses to the 13 nitro compounds from Figure 7.6. The first three principal component scores are plotted on three axes; data from eight nanotube species was used.

Table 7.1: Loadings for the first three principal components listed by input variable. The PCA inputs consist of the intensity and wavelength changes for eight SWNT species.
Figure 7.8: Nitro group-containing compounds exposed to bombolitin II-SWNT.
7.4 MECHANISM OF RDX DETECTION

The bombolitin-SWNT response to RDX is dependent on the peptide sequence. Aqueous solutions of peptide-stabilized SWNT were made with three of four members of the bombolitin family. All are amphiphilic, helical, 17-residue peptides isolated from bumblebee venom (See methods for sequences). The bombolitin I variant failed to solubilize nanotubes while bombolitins II, III, and IV made highly NIR photoluminescent, stable solutions via probe-tip sonication in the presence of SWNT (Figure 7.9a-c). The NIR spectra of the solubilized nanotubes show variations in relative initial emission intensities and wavelengths of the PL maxima of the \((n,m)\) SWNT species present in the sample. Upon introduction of 90 uM RDX, the photoluminescence peaks of bombolitins II and III undergo distinct red-shifts of up to 13 meV, with the magnitude dependent on the \((n,m)\) species (Figure 7.9a-b). The SWNT solubilized by bombolitin IV, however, exhibits no wavelength shifting behavior (Figure 7.9c). Circular dichroism (CD) measurements conducted on the peptides in the absence of nanotubes show similar spectra for bombolitins II and III, but the bombolitin IV spectrum differs, more closely resembling the classical alpha helical spectrum (Figure 7.9d-f). Upon introduction of RDX, the bombolitin II and III spectra exhibit a distinct blue shift of their 206 nm peak and significant increase in the negative ellipticity. The bombolitin IV spectrum, upon the same treatment with RDX, exhibits negligible change. This data suggests a specific binding of RDX to the bombolitin II and III peptides, inducing a conformational change upon binding. The binding is sequence specific, as bombolitin IV shows little conformational change upon exposure to RDX.
Figure 7.9: Photoluminescence spectra of peptide-suspended nanotubes before (blue) and after (red) addition of 90 µM RDX. (a) Spectra of nanotubes suspended by bombolitin II (b) bombolitin III (c) bombolitin IV. Circular dichroism spectra of the same peptides in the absence of nanotubes before (blue) and after (red) addition of RDX. (d) CD spectrum of bombolitin II, (e) bombolitin III, (f) bombolitin IV.
The wavelength shifting of bombolitin II-solubilized SWNT exhibits concentration dependence which fits well to a first-order Langmuir adsorption isotherm (Figure 7.10), implying that the transition is reversible. The bombolitin II peptide is found to exhibit low affinity to SWNT, as it can be dialyzed away, causing aggregation of the nanotubes, evident from PL quenching in Figure 7.11. This behavior suggests that the nanotube reports the conformation of the largely freely-suspended peptide. The nanotube, although solubilized by the peptide, acts as a “chaperone sensor,” which indirectly detects the binding event by transducing changes to the native conformation of the bombolitin. We introduce the term because it is the first such nanoscale sensor of its type.

![Figure 7.10](image_url)

**Figure 7.10:** Center wavelength of the (11,3) nanotube peak of bombolitin II-solubilized SWNT plotted versus RDX concentration (red circles). The data is fit to a first-order Langmuir adsorption isotherm (blue curve).
Figure 7.11: Photoluminescence spectrum of bombolitin II-solubilized SWNT before (blue) and after (pink) dialysis using a 20,000 MWCO membrane through which the free peptide, but not the nanotubes, can pass. Quenching of the PL denotes nanotube aggregation in the absence of other external factors.

As seen in Figure 7.9, different nanotube species do not respond equally to RDX-induced conformational changes of the peptide. Certain \((n,m)\) species are more sensitive to RDX interrogation, and others exhibit a greater total degree of wavelength shifting. The Langmuir equilibrium constant, \(K\), of RDX binding varies by nanotube species. The highest \(K\)'s found were above 0.12 \(\mu\)M, on the order of the \(K_d\) of a typical high affinity peptide binding interaction (for example, 0.11 \(\mu\)M for a VEGF-binding peptide\(^{23}\)). The maximum wavelength shift \((\Gamma_{\text{max}})\), exhibits a different dependence on SWNT species (Figure 7.12). The \(\Gamma_{\text{max}}\) is found to reach a maximum when the SWNT species diameter and chiral angle are greatest. Such a relationship would follow if the bombolitin II, in its RDX-bound conformation exhibited poorer stabilization of the larger, more chiral nanotubes as compared to the smaller SWNT with lower chiral angles.
Figure 7.12: Langmuir isotherm parameters of individual SWNT species from RDX-induced shifting of bombolitin II-encapsulated SWNT. Langmuir equilibrium constant (top) and maximum wavelength shift (bottom) of the responses of eight nanotube species plotted versus nanotube chiral angle and diameter.

The native conformation of the bombolitin II peptide in aqueous solution is largely unordered although some structure exists given the broad shoulder at 222 nm in
the circular dichroism spectrum. Upon introduction of low concentrations of sodium
dodecyl sulfate (SDS) to the peptide, β-aggregates form in solution. At
concentrations near the critical micelle concentration (CMC), the peptide suspends in
solution and adopts an α-helical conformation. This was demonstrated in the presence of
the nanotube by addition of SDS to the bombolitin II-SWNT suspension (Figure 7.13).
Upon addition of 80 µM SDS to 45 µM peptide, the nanotube PL falls and shifts
dramatically, and precipitates are visible. This compares favorably with CD data in the
literature which shows a distinct change in ellipticity of the peptide perturbed with SDS
at the same concentration ratio. The SWNT intensity increases and blue-shifts at higher
SDS concentrations, but it is not clear whether this was caused by the re-suspension of
the peptide or the direct suspension of nanotubes by SDS. Regardless, this experiment
demonstrates the high sensitivity of nanotube PL to the bombolitin II conformation.
Figure 7.13: Bombolitin II-SWNT photoluminescence changes upon addition of SDS. Variations in the intensity (top) and wavelength (bottom) of the (6,5) species after one hour of equilibration in a well plate. (Error bars indicate one standard deviation.)
7.5 SINGLE-MOLECULE ANALYTE DETECTION

We have constructed a near-infrared dual-channel microscope for imaging spectral shifts of nanotube PL (Figure 7.14). The microscope allows spectroscopic information to be elucidated by splitting the image into two channels which are adjacent in wavelength. The light from one nanotube emission band is split into two beams. The light from both beams is treated with filters to spectroscopically isolate one emission peak, and then to permit only half of the peak’s emission to appear in each channel. One channel on the same near-infrared detector array shows the long wavelength half of the peak, and the other contains the short wavelength half. The filters used were designed to measure the (7,5) nanotube. The 50% cut-off/cut-on of the edge filters were at 1030 nm. A 1000 longpass and 1100 nm shortpass filter were placed in the emission beam before splitting to isolate the (7,5) peak and block all other SWNT emission.

This microscope detects minute SWNT spectral shifts which signify RDX binding to bombolitin II. Nanotubes are immobilized on a glass surface and imaged in the presence of 8 µM of the peptide in Tris buffer. The emission of each PL spot in the detector is binned in a 2x2 pixel area and measured in each channel over the course of a 2000-second movie taken at one frame per second. The RDX is introduced to the peptide solution above the immobilized nanotubes during the course of the movie. The traces were fit by a hidden Markov algorithm. The long-wavelength nanotube emission responds by exhibiting distinct upward steps, while the short-wavelength SWNT emission exhibits downward trends. This anti-correlated behavior denotes red-shifting of the surface-bound nanotubes’ photoluminescence. Correlated downward steps denote
quenching of the exciton. An example pair of traces from the two channels (Figure 7.15) exhibit both correlated and anticorrelated behavior after introduction of RDX.

Histograms of compiled upward and downward steps from time traces of 13 nanotubes exposed to the aforementioned conditions yield a narrow shape with a maximum normalized intensity near 0.2, signifying a discrete step height corresponding to a quantized intensity (Figure 7.16). Such a distribution suggests that single molecules of RDX binding to the peptide, as well as un-binding events, are detectable by this method. A slightly wider distribution of the short WL channel’s step heights is attributable to the fast quenching response apparent just after RDX perfusion, which is likely to cause several near-simultaneous events which occur within the time resolution of the experiment.

Later un-correlated events due to quenching steps present in the long WL channel are due to the RDX-induced quenching of the emission which has already shifted away from the short WL channel and therefore exhibits low relative intensity in that channel. This is evident by plotting the absorption spectra of the edgepass filters with the SWNT PL spectra before and after RDX addition (Figure 7.17).
Figure 7.14: Near-infrared dual-channel microscope. Light leaving the sample passes through a bandpass or two edgepass filters to spectroscopically isolate one fluorescence band. This light reaches a 50/50 beamsplitter which creates two equal beam pathways. The resulting beams pass through edgepass filters (a longpass for one and a shortpass for the other) with a 50% cut-off or cut-on at the same wavelength. The beams are captured by two different regions of a near-infrared array detector. The image shows the same location of the microscope field on both the Short WL and Long WL channels, but the spectral region is different. The two red boxes encircle the same nanotube in both channels.
**Figure 7.15**: Time trace of the intensity of the same nanotube’s photoluminescence in the short WL (green) and long WL (blue) channels fit by a hidden Markov algorithm. 90 μM RDX was added at 100 s.

**Figure 7.16**: Histograms of normalized step sizes from fitted time traces of 13 different nanotubes upon RDX addition.
7.6 CONCLUSIONS

We have demonstrated the detection of several nitro compound-containing explosives and pesticides via redox bleaching and solvatochromic shifting of carbon nanotube photoluminescence. Selective sensing of the explosive TNT is detected by charge transfer to a DNA-encapsulated nanotube. The explosives RDX, and picric acid, as well as pesticides TFM and 2,4-dinitrophenol are detected by conformational changes of SWNT encapsulated by a single oligopeptide sequence. The nanotubes detect binding of the explosive RDX to the peptide by their sensitivity to the peptide conformation, creating a reversible, real-time sensor. Due to the unique responses of eight SWNT species’, the responding analytes can be identified. The work conclusively demonstrates that polymers and biopolymers which encapsulate SWNT can determine sensor properties, including analytes detected, sensitivity, and selectivity.
7.7 EXPERIMENTAL METHODS

7.7.1 Preparation of SWNT suspensions

Single-walled carbon nanotubes synthesized by the HiPCO method (Unidym) were suspended in a 2:1 mass ratio of Bombolitin to SWNT in 20mM Tris and 100 mM NaCl (henceforth known as Tris buffer) using a 1/8th inch probe-tip sonicator (Vibra-Cell) at 10W for 10 min. The resulting bombolitin-SWNT solution was centrifuged twice for 90 minutes at 16,300g and the pellet removed each time. Bombolitin sequences used were bombolitin I: IKITMLAKLGKVLAV, bombolitin II: SKITDILAKLGKVLAV, bombolitin III: IKIMDLAKLGKVLAV, and bombolitin IV: INIKDILAKLVKVLGHV (AnaSpec).

DNA-encapsulated SWNT was prepared by sonicating nanotubes in the presence of d(GT)\textsubscript{15} DNA (IDT) in a 2:1 DNA:SWNT ratio in a solution of 0.1 M NaCl using the same sonication and centrifugation steps as above.

Suspensions of SWNT encapsulated by PVA were prepared by first sonicating nanotubes in the presence of a 2% aqueous solution of sodium cholate for 20 minutes in a 750W cup-horn sonicator (Vibra-Cell) at 90% amplitude. Suspensions were ultracentrifuged for 4h at 100,000g and the pellet removed. The resulting suspension was stirred with 2% PVA (31,000-50,000 MW) overnight at 70 °C then dialyzed against 2L of water for 24h.

7.7.2 Preparation of RDX solutions

A mixture of 10:1 weight ratio sand to RDX was added to acetonitrile and filtered through a 0.45 micron syringe filter. The resulting solution was precipitated in excess
water and filtered using a 0.22 micron millipore filter using a vacuum flask. The filtered solid RDX was dissolved in acetonitrile as a stock solution. An aqueous solution was prepared by adding 1% stock solution in acetonitrile to water and centrifuged twice at 16,300g for 5 minutes and the pellet removed each time.

7.7.3 Analyte Screening and Spectroscopy

Analyte screening was conducted in a 96 well plate containing either a bombolitin-SWNT solution of 8uM peptide or a PVA-SWNT solution of 2mg/L SWNT. The SWNT solutions were interrogated by the analytes added separately to each well. The nanotubes and analytes were incubated for 1 hour. Near-infrared photoluminescence spectra were obtained using 785nm excitation and an Acton SP-150 spectrograph coupled to a Princeton Instruments OMA V InGaAs detector. Bombolitin-SWNT prepared alternately with peptides bombolitin II, III, and IV and mixed with 90uM RDX in 1% acetonitrile solution. The near-infrared photoluminescence spectra were obtained 1h after mixing.

7.7.4 Circular Dichroism.

Circular Dichroism measurements were obtained using an Aviv Model 202 Circular Dichroism Spectrometer in a 1mm pathlength strain-free cuvette. Bombolitin solutions of 0.275mM in Tris buffer were measured before and after the addition of 9nM RDX solution in 1% acetonitrile while keeping the bombolitin concentration constant.
7.7.5 SDS Addition Experiment

Bombolitin-SWNT solutions containing 44 µM bombolitin II in Tris buffer were aliquoted in a well plate and mixed varying concentrations of SDS. Near-infrared photoluminescence spectra were acquired 1h after mixing.

7.7.6 Microscopy of SWNT Solvatochromic Shifts

As-produced Bombolitin II-SWNT was incubated on a glass coverslip-bottom petri dish (MatTek Corporation) for 30 minutes and subsequently rinsed 3x with Tris buffer. The glass surface was then covered with 100 µL Tris buffer including 8µM of bombolitin II peptide. An aliquot of 100 µL of 180 µM RDX suspended in Tris buffer was added to the petri dish 100 seconds after data collection began. The glass surface was imaged by exciting with a 658 nm laser on a Zeiss Axio Observer D1 microscope whose light path was modified by the optical setup illustrated in Figure 7.10 after the light passed through a 5 mm slit. Movies were collected at 1 frame/second. The movies were processed by averaging the signal over a spot size of 2x2 pixels and plotting the resulting intensity versus time. A SWNT photoluminescence spot on the left channel correlates to the one on the right channel by having the same y-axis value and being 160 pixels apart in the x-axis. Time traces of the average intensity of the 2x2 pixel spots were normalized to their initial values.

7.8 LIST OF REFERENCES


CHAPTER 8:  
CONCLUSIONS AND FUTURE DIRECTIONS

The state of the field of carbon nanotube-based optical sensing at this point is a promising one. Nanotubes have been shown to possess properties including photostability and high sensitivity that could lead to sensors with unique advantages over current technologies. The work presented herein and continued by colleagues demonstrates nanotube-based sensors which detect an increasing number of analytes including metal cations, chemotherapeutic drugs, glucose, hydrogen peroxide, singlet oxygen, hydroxyl radicals, and others. The various sensors allow unprecedented capabilities such as single-molecule sensitivity, real-time detection in live cells, sensing through tissues, including whole blood, signal multiplexing, and analyte identification.

Current challenges to widespread use of such technologies include the availability, cost and ease-of-use of near-infrared cameras and spectrometers, as well as issues of sample preparation and measurement, which vary depending on detection conditions and goals. To develop this into a viable technology, one must be cognizant of limitations such as the requirement that nanotubes can only detect changes which occur to their surface. While detection of such interactions such as protein-protein and small molecule-protein may be possible, transduction must occur through a change that perturbs the electronic structure of the nanotube.

Despite these difficulties, recent work is moving nanotube-based optical sensors towards real applications. The single-molecule stochastic sensing of hydrogen peroxide, highlighted in Chapter 6, has been used by colleagues to detect real-time excretion of the molecule from live cells spatially. This type of nanotube-based optical sensor has the potential to be an important research tool due to its sensitivity and ability to transiently...
detect bioanalytes. A recent discovery, detection of nitric oxide, has been demonstrated from within a whole animal, permitting in vivo sensing of an important biological signaling molecule. Current work has demonstrated single-molecule detection of explosives.

Due to the promises of high sensitivity and other unique capabilities of nanotube-based optical sensors, the author anticipates that this work will reach fruition in the development of transformative technologies of interest to researchers and the general public.
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2010 - Postdoctoral Research Fellow, Department of Chemical Engineering, Robert Langer Group, Massachusetts Institute of Technology, Boston, Massachusetts
2006 Visiting Scientist/CESRI Fellow, Jörg Langowski Group, German Cancer Research Center, Heidelberg, Germany
2003-2009 Graduate Researcher, Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois
2002-2003 Visiting Scientist, Rice Quantum Institute, Robert Curl Group, Rice University, Houston, Texas
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2002-2003 Researcher, Visigen Biotechnologies, Houston, Texas

Education

2003-2010 Ph.D. in Chemistry, University of Illinois at Urbana-Champaign, 2009

Awards and Honors

2009 Finalist, Materials Research Society Graduate Student Awards
2009 Poster Award, Tenth International Conference on the Science and Application of Nanotubes, Beijing, China
2008 1st Place, Bionanotechnology Graduate Student Award Session, American Institute of Chemical Engineers Annual Meeting
2008 2nd Place, Nanoscale Science & Engineering Forum Poster Session Award, American Institute of Chemical Engineers Annual Meeting
2007 Oral Presentation Award, 6th Annual Chemical and Biomolecular Engineering Symposium, University of Illinois
2007 3rd Place, Center for Nanoscale Science and Technology Poster Award, University of Illinois
2006 Walter Brown Fellowship, Department of Chemistry, University of Illinois
2006 2nd Place, Cell and Molecular Biology and Molecular Biophysics Training Grants 19th Research Symposium Poster Award, University of Illinois
2006 Poster Award, 5th Annual Chemical and Biomolecular Engineering Symposium, University of Illinois
2006 Hanratty Travel Award, Dept. of Chem. and Biomolecular Eng., University of Illinois
2006 Beckman Institute Graduate Fellowship, University of Illinois
2006 Physical Chemistry Poster Session Award, Department of Chemistry, Univ. of Illinois
2006  Collaboration Success Award, Council of Chemical Research
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2006  Graduate College Travel Award, University of Illinois
2003  Roger Adams Fellowship, Department of Chemistry, University of Illinois

Publications List

Journal Publications


**Book Chapters**


**Patents**


**Invited Talks**


**Other Lectures**

“Single-Walled Carbon Nanotubes for Optical Biosensing with Multiple Modes.” 2nd Carbon Nanotube Biology, Medicine & Toxicology Satellite Symposium, Tenth International Conference on the Science and Application of Nanotubes, Beijing, China, June, 2009.


**Leadership and Outreach**

2009-present  *Teacher/Volunteer*, MIT Museum, Cambridge, Massachusetts
2006-2008  *Founder and President*, Center for Nanoscale Science and Technology Student Initiative, University of Illinois
2006-2008  *Founder and Organizer*, Graduate Seminar in the Applied Chemical Sciences, School of Chemical Sciences, University of Illinois
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2003-2004  *Organizer*, Careers in Academia Seminar Series, Department of Chemistry, University of Illinois
1996-2003  *Teacher*, Astronomy and Education Departments, Houston Museum of Natural Science, Houston, Texas

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2008-present  *Mentor*, 4 Undergraduate Research Opportunities Program Scholars, MIT
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2003-2008  *Mentor*, 8 Undergraduate Research Assistants, University of Illinois
2003-2004  *Graduate Teaching Assistant*, University of Illinois Chem 347 “Physical Chemistry Laboratory,” Teaching Assistant
2000-2002  *Science Teacher, 7th and 8th grades*, The Kinkaid School, Houston, Texas

**Professional Associations**

American Chemical Society
American Institute of Chemical Engineers
Materials Research Society
American Physician Scientists Association