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SINGLE-WALLED CARBON NANOTUBES FOR ADVANCED OPTICAL
MATERIALS, WEARABLE BIOSENSING, AND BIOIMAGING

BY

MOHAMMAD MOEIN SAFAEE

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
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OF
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2020

Abstract

Single-walled carbon nanotubes (SWCNTs) functionalized with synthetic or natural polymers are a class of advanced optical nanomaterials that have recently found applications in a wide range of molecular diagnostics platforms as biosensors and bioimaging agents. Despite their widespread applications, there is an urgent need to develop standard characterization assays to evaluate their optical, physical, and interfacial properties *in vitro* and in complex biological environments. The results from such assays are essential for defining general design principles for nanotube-based optical probes and biosensing devices. Moreover, to fully exploit the nanotube's unique properties for health monitoring, it is essential to employ micro- and nano-fabrication processes to produce bulk or thin-film optical materials capable of real-time biomarker monitoring for wearable and implantable biosensing applications.

In this dissertation, we developed an entirely experimental platform based on time-resolved near-infrared fluorescence spectroscopy, hyperspectral fluorescence microscopy, and confocal Raman imaging, among other experimental approaches, to investigate the optical and hybrid stabilities of DNA-wrapped SWCNTs (DNA-SWCNTs) in solution phase and in live cells. We systematically quantified the time constant of the DNA displacement as a measure for the DNA-nanotube hybrid stability through a surfactant exchange mechanism and measuring the resultant fluorescence response. The stability was demonstrated to directly correlate with the DNA sequence length, although no statistical correlation was observed between the stability and optical parameters, indicating that the stability is not a result of the surface coverage afforded by DNA. To translate these findings into biological environments, utilizing hyperspectral fluorescence and confocal Raman

imaging, we showed that a longer DNA length leads to a greater relative cellular uptake, intracellular optical stability, and retention of DNA-SWCNTs in mammalian cells. Additionally, by labeling the DNA with a fluorophore that dequenches upon removal from the SWCNT surface, we found that shorter DNA strands are displaced from the SWCNT within the cell, altering the physical identity and biocompatibility, thus changing the fate of the internalized nanomaterial.

Next, we employed atomic force microscopy (AFM) and thermogravimetric analysis (TGA) to investigate the physical and material characteristics of the DNA-nanotubes, namely their length distribution, DNA coverage density, and thermal stability, as a function of the DNA sequence. We discovered that the DNA coverage densities and conformations on the nanotubes significantly differ by varying the DNA sequence length. Consequently, this introduces an overlooked artifact to the electrostatic repulsions between the hybrids and the imaging substrate. Thus, we developed a modified standardized protocol for AFM-based size quantification by suppressing the wrapping and free diffusion effect so that the deposited hybrids represent their parent solutions. Moreover, the DNA conformations revealed in this study confirmed the instabilities of the short DNA sequences in the biological environment. Utilizing TGA, for the first time, we also discovered that the thermal stability of the purified nanotubes can be substantially enhanced upon hybridization with DNA, and their thermal decomposition behavior can be manipulated by modulating the bases in the DNA sequence.

Finally, we introduced the first generation of the nanotube-based optical wearable textiles by encapsulating the DNA-nanotube hybrids inside of core-shell microfibers without altering their unique nanoscale optical properties. We optimized a robust

fabrication process that can be applied to a wide range of water-soluble polymer-wrapped nanotube biosensors. Utilizing probe fluorescence spectroscopy and confocal Raman microscopy, we demonstrated that the microfibers maintain their optical stability and structural integrity for up to 21 days without releasing the nanotube biosensors into their surrounding environment. We ultimately designed and calibrated a smart wound dressing for continuous wireless monitoring of oxidative stress in the wound environment.

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Thank you!

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Preface

This dissertation is written in manuscript format and includes seven chapters. Chapter 1 presents an introduction to the main topics and methods included in this dissertation. Chapter 2 entitled “DNA–Carbon Nanotube Complexation Affinity and Photoluminescence Modulation are Independent” was published in ACS Applied Materials and Interfaces (*ACS applied materials & interfaces* **2017**, *9*, 21397-21405) in June 2017. Chapter 3 entitled “Biomolecular Functionalization of a Nanomaterial to Control Stability and Retention within Live Cells” was published in Nano Letters (*Nano letters* **2019**, *19*, 6203-6212) in August 2019. Chapter 4 entitled “DNA Sequence Mediates Apparent Length Distribution in Single-Walled Carbon Nanotubes” was published in ACS Applied Materials and Interfaces (*ACS applied materials & interfaces* **2018**, *11*, 2225-2233) in December 2018. Chapter 5 entitled “Enhancing the Thermal Stability of Carbon Nanomaterials with DNA” was published in Scientific Reports (*Scientific reports* **2019**, *9*, 1-11) in August 2019. Chapter 6 entitled “A Wearable Optical Microfibrous Biomaterial with Encapsulated Nanosensors Enables Wireless Monitoring of Oxidative Stress” is under review in Advanced Functional Materials. Chapter 7 presents concluding remarks and future directions for this dissertation. Appendices 1, 2, 3, 4 and 5 contain supporting information for chapters 2, 3, 4, 5 and 6, respectively.

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Chapter 1. Introduction

1. 1. An Introduction to Single-Walled Carbon Nanotubes

Over the past three decades and especially from the beginning of the 21st century, synthesis, fabrication, functionalization, and manipulation of materials in the nanoscale have been the objective of various disparate research fields to create novel materials with extraordinary properties for a wide range of applications from therapeutics to electronics.¹⁻⁴ Single-walled carbon nanotubes (SWCNTs), categorized as 1D carbon nanomaterials, have attracted substantial attention among various scientific communities owing to their unique intrinsic optical, chemical, and mechanical properties.⁵ The electronic bandgap energies of SWCNTs result in various semiconducting species (chiralities) which exhibit a distinct narrow bandwidth photoluminescence (fluorescence) in the second near-infrared (NIR-II) window.^{6, 7} The SWCNT fluorescence responds to their local environment, resulting in shifts in emission wavelengths and/or variations in intensity; thus they can function as advanced biomolecule sensing devices.⁸ Moreover, the presence of aromatic rings and delocalized π electrons on the outer wall of the SWCNTs facilitates conjugating therapeutic and targeting moieties to their surface through $\pi - \pi$ stacking to design multimodal imaging, sensing, and delivery agents in complex biological environments.⁵ In addition to their exceptional optical and chemical properties, SWCNTs have also been reported to possess extraordinary mechanical properties, notably an extraordinary Young's modulus⁹ and flame-retardant characteristics,¹⁰ which make them promising candidates as structure-supporting and strength-reinforcing agents for materials engineering applications. An initial essential step for utilizing the SWCNTs in many of these

applications is to prepare stable colloidal dispersions of these nanomaterials in an aqueous environment by functionalizing them with amphiphilic polymers, biomolecules, or surfactants.^{5, 11} To design biosensing devices, bioimaging agents, and advanced composite materials based on the functionalized SWCNTs, a thorough characterization of their fundamental properties is critical. Additionally, the development of robust fabrication processes is crucial to produce devices incorporating SWCNTs without altering their intrinsic electronic and optical properties. Utilizing various imaging and spectroscopy modalities, we explore the fundamental characteristics of the functionalized SWCNTs and fabricate the first generation of wearable biosensing devices based on the fluorescent SWCNTs.

1. 2. SWCNT Structure and Photophysics

SWCNTs are composed of a single graphitic layer rolled up into a one-dimensional nanocylinder with diameters ranging from less than 1 nm to a few nanometers (Figure 1. 1). The roll-up direction of the graphitic layer can be defined by a vector called chiral vector.⁷ The chiral vector can be decomposed to two vector components $n\vec{a}_1$ and $m\vec{a}_2$, and can be defined by its scalar components, n and m , as follows:

$$\text{Chiral Vector } (n, m) = n\vec{a}_1 + m\vec{a}_2$$

Based on the vector geometry principles, the diameter of the nanotubes can be derived based on the scalar components of the chiral vector and the Carbon-Carbon single bond length in hexagonal graphitic lattice⁵:

$$d = \frac{\sqrt{3}}{\pi} d_{C-C} \sqrt{n^2 + nm + m^2}$$

In addition, the chiral angle (θ) ranges from 0 to 30 degrees. SWCNTs with chiral

angle of 0 and 30 degrees are called zigzag and armchair structures, respectively.¹²

$$\theta = \tan^{-1} \left(\frac{\sqrt{3}m}{2n+m} \right)$$

Most importantly, the electronic properties of the SWCNTs, namely bandgap energies and metallic or semiconducting behavior, vary based on the chiral vector and the SWCNT diameter.^{5, 7, 12} Thus, rolling up the graphene sheet with different chiral vectors results in distinct chiralities of the nanotubes. In zigzag $(n, 0)$ SWCNTs, if $n/3$ is an integer, the nanotube is metallic and otherwise it is a semiconductor. In (n, m) chiral SWCNTs, if $(2n + m)/3$ is integer, the SWCNTs are metallic and else they are semiconductor. Finally, the armchair (n, n) SWCNTs are metallic.⁵

Figure 1. 1. Schematic of the graphene sheet segment showing the indexed lattice points. Nanotubes corresponding to (n, m) are obtained by rolling the sheet from $(0,0)$ to (n, m) along a roll-up (chiral) vector.¹³

As a result of the quantum confinement along the radial direction of the SWCNTs,^{6, 7, 14} all SWCNT chiralities possess extremely sharp maxima of electronic density of states,

called Van Hove singularities, in their energy band diagrams (Figure 1. 2a and b), however, only semiconducting SWCNTs feature a chirality-dependent fluorescence behavior upon photon absorption because their density of states is zero at the Fermi level (Figure 2b).¹⁵ Figure 1. 2c and d demonstrate various semiconducting SWCNT chiralities and their corresponding emission spectra.

Figure 1. 2. (a) Band diagram of metallic SWCNTs, showing no fluorescence emission due to the continuous density of states near the Fermi level.⁵ (b) Band diagram of semiconducting SWCNTs, showing fluorescence emission after excitation.⁵ (c) Semiconducting SWCNT chiralities false-colored to demonstrate their distinct emission wavelengths. (d) The corresponding fluorescence emission spectra of the semiconducting SWCNTs.¹⁶

The photon absorption and photoluminescence emission in SWCNTs is an

extremely fast process which follows a multi-step mechanism.⁵ The process starts when SWCNTs absorb a photon with the photon energy matching the bandgap of the E_{22} transition that is the difference in energy between the second valence band (v_2) and the second conduction band (c_2).^{5, 7, 17} Following the photon absorption, an electron is excited; thus it moves from the second valence band to the second conduction band and leaves a hole behind. As the electron and hole are oppositely charged, they remain bound to each other and form a pair called exciton.^{5, 18} The exciton binding energy is usually around 400 meV for semiconducting SWCNTs.⁵ After the exciton formation, both the electron and the hole non-radiatively relax to the lowest energy level in both valence and conduction bands, i.e., the v_1 and c_1 bands.¹⁸ Finally, the exciton travels along the length of the nanotube through diffusion until the electron and hole recombine radiatively to give off a fluorescence photon with its energy matching the bandgap across E_{11} transition minus the exciton energy.¹⁹ Alternatively, the exciton can release its energy through a non-radiative Auger process if it encounters a chemical sidewall functionalization or defect, resulting in fluorescence quenching.¹⁹

The fluorescence emission from the SWCNTs possesses four unique properties which make them ideal candidates for a wide range of applications:

First, the photon absorption by SWCNTs mostly occurs in the visible (400–750 nm) and NIR-I (750–1000 nm) windows, followed by fluorescence emission in the NIR-II window (1000–1700 nm) and non-radiative relaxation in the form of heat.⁵ Due to their low absorption, reduced scattering, and minimum autofluorescence from biological tissues, NIR-I and NIR-II windows are biologically transparent and ideal for bioimaging applications.⁵ Moreover, since the Stokes shift between the absorption and emission

windows can be up to several hundreds of nanometers, the excitation light does not spectrally overlap with the emission window.²⁰ This feature significantly facilitates designing imaging and spectroscopy instruments for the SWCNTs.

Second, due to the excitonic nature of the fluorescence signal from the SWCNTs and given the fact that every individual carbon atom on their surface is exposed to their surrounding environment, their fluorescence emission responds to their local environment through exciton quenching, Fermi level, or solvatochromic shifting.⁸ This environmental sensitivity makes the SWCNTs ideal candidates for various biosensing applications.

Third, their chirality-dependent fluorescence enables simultaneously designing wavelength-specific probes for multiple analytes, thereby enabling synthesis of multiplexed or ratiometric analyte imaging and sensing platforms.^{16, 21}

Forth, as the SWCNTs do not structurally degrade under standard biological conditions, they exhibit robust photostability that allows long-term bioimaging and biosensing without blinking and signal loss.^{22, 23}

1. 3. SWCNT Surface Functionalization

Due to their hydrophobic surface made of sp^2 carbon atoms, water solubility, long-term colloidal stability and biocompatibility are three characteristics that are essential to be introduced to the pristine SWCNTs through amphiphilic molecular functionalization, to enable their biomedical applications.⁵ In addition to these, introducing molecular recognition features to the surface of the SWCNTs is crucial to target them to specific biomarkers and receptors for drug delivery, bioimaging and biosensing applications.¹¹ The available large surface area of the SWCNTs, comprised of sp^2 carbon atoms, facilitates

their covalent and non-covalent functionalization to simultaneously impart water solubility, biocompatibility and molecular recognition to the individual SWCNTs.⁵

1. 3. 1. Covalent Surface Functionalization

Covalent surface functionalization is performed by introducing heteroatoms (mostly N and O) and functional groups to the pristine SWCNTs and thus enhances the water solubility.⁵ The functional groups also provide further active sites for conjugation with other functional molecules to improve biocompatibility and impart molecular recognition moieties to the original SWCNTs.^{24, 25} Over the past 20 years, various conventional covalent functionalization strategies have been developed for SWCNTs and other carbon nanomaterials. For instance, controlled oxidation in the presence of various oxidizing agents such as KMnO_4 , H_2SO_4 , and HNO_3 is a widely accepted methodology for covalent functionalization of the SWCNTs.²⁵ Moreover, alkyl functionalization,²⁶ cycloaddition to azomethine for pyrrolidine functionalization²⁷, and EDC- catalyzed amidation to conjugate proteins and polymers through their $-\text{NH}_2$ groups to the $-\text{COOH}$ groups on the nanotubes^{28, 29} are the other widely utilized strategies for the covalent functionalization of SWCNTs. The broad range of solubilization and conjugation approaches provided by covalent methods is a great advantage; however, the damage induced by these methods to the conjugated π network results in non-radiative recombination of excitons at the defect site and decreased quantum efficiency.⁵ Nonetheless, several novel covalent functionalization processes have recently been developed to preserve or enhance the fluorescence emission of the SWCNTs. Defect engineering is one of the strategies that can significantly enhance their NIR-II fluorescence

emission by introducing controlled sp^3 defect sites into semiconducting SWCNTs.³⁰ Although the sp^3 defect sites can be utilized as anchor points for peptide and protein conjugation,³¹ the conjugation of various types of biomolecules to the defect anchor points can sometimes be challenging due to the limitations in modulating the defect chemistry. Therefore, a universal covalent functionalization strategy has been developed that both preserves the SWCNT NIR-II fluorescence and enables conjugating many different functionalities to their surface.^{32, 33} This novel strategy re-aromatizes defect sites to restore the original sp^2 surface of the SWCNTs and their intrinsic fluorescence. This development has enabled synergistic combination of covalent and non-covalent functionalization strategies to impart multiple functionalities to SWCNTs, namely water solubility, biocompatibility, and stable molecular recognition ability.³²

1. 3. 2. Noncovalent Surface Functionalization

Non-covalent functionalization involves supramolecular interactions, specifically $\pi - \pi$ stacking or hydrophobic interactions between the SWCNT surface and the hydrophobic segments of the amphiphilic molecules, that impart minimum structural damage to the sp^2 lattice of the SWCNTs, resulting in preserving their NIR-II fluorescence.⁵ Non-covalent functionalization can usually be implemented by ultrasonication of the SWCNTs in the presence of certain amphiphilic molecules such as surfactants, short single-stranded DNA (ssDNA),^{34, 35} and synthetic polymers.¹¹ Under ultrasonication, the hydrophobic and van der Waals interactions between the hydrophobic segments of the amphiphilic molecules and SWCNTs overcome those between bundled nanotubes, resulting in individually dispersed surfactant- or polymer-wrapped SWCNTs⁵

(Figure 1. 3) that have a lower density than the bundled SWCNTs and catalyst particles. Taking advantage of this density difference and utilizing ultracentrifugation, the individually dispersed SWCNTs can be separated from the remaining large bundles, aggregates, and catalyst particles.

Figure 1. 3. Schematic demonstrating a pristine SWCNT and an amphiphilic polymer. Ultrasonication of the pristine SWCNTs in presence of the amphiphilic polymer yields individually dispersed polymer-wrapped SWCNTs in aqueous environments.¹¹

In addition to preserving the unique optical properties of the SWCNTs, the other imperative advantage of the non-covalent functionalization using ssDNA and other polymers is that they can both solubilize the SWCNTs in water and act as artificial antibodies, imparting molecular recognition pockets on the SWCNT surface.¹¹ The phenomenon whereby a polymer corona phase wrapped around the SWCNTs selectively recognizes a particular molecule is called corona phase molecular recognition (CoPhMoRe)¹¹ (Figure 1. 4). By wrapping the SWCNTs in libraries of natural or synthetic polymers and screening their fluorescence response to a particular target biomolecule, a targeted biosensor, bioimaging agent, or drug carrier can be identified without using expensive recognition moieties like antibodies and performing complicated conjugation

chemistries.¹¹

Figure 1. 4. Schematic illustrating the molecular interaction of an analyte with the corona phase of a SWCNT, which can result in either intensity or wavelength modulation of the SWCNT fluorescence.¹¹

DNA is particularly important for SWCNT non-covalent functionalization as it is one of the most fundamental biological molecules that has been thoroughly studied since the discovery of its structure.³⁶ ssDNA, particularly, can be synthetically fabricated at high yield and purity in lengths up to 100 nucleotides. Within a single nucleotide, there exist hydrophilic (phosphate backbone) and hydrophobic regions (aromatic bases). Therefore, ssDNA is an amphiphilic molecule and thus has associated surface acting properties. In particular, the 1D polymeric structure of the ssDNA and its hydrophobic backbone allows the molecule to efficiently solubilize the SWCNTs in aqueous environments upon ultrasonication.³⁶ Atomic force microscopy (AFM) images and molecular dynamics (MD) simulations have indicated a helical conformation of ssDNA while adsorbed onto the SWCNT (Figure 1. 5a and b).^{37, 38} The final hybrid nanomaterial is called an ssDNA-wrapped SWCNT or ssDNA-SWCNT. Of note, the 3D structure of the ssDNA corona on

SWCNTs has been demonstrated to depend on both ssDNA sequence and SWCNT chirality,³⁷ enabling designing various targeted biosensors and bioimaging agents selective to a wide range of chemical disease biomarkers including lipids,³⁴ neurotransmitters,³⁹ and reactive oxygen species.⁴⁰ Moreover, the ssDNA has been demonstrated to be extremely promising for the purification of single chirality SWCNTs. Over the past decade, libraries of recognition short ssDNA oligomers (10-20 mers) have been identified that select and enrich to high purity one particular chirality of SWCNTs upon ion-exchange chromatography (IEX) or aqueous two-phase separation (ATP).⁴¹ For instance, the ssDNA sequence (TAT)₄ will enrich the (6,5)-SWCNT to 90% purity from a mixture of multiple chiralities.³⁷

Figure 1.5. (a) Schematic and (b) AFM image of an ssDNA-SWCNT. The bright spots in the AFM image each indicate a pitch of ssDNA molecule around the SWCNT.

1. 4. Optical Microscopy and Spectroscopy Approaches for SWCNT Characterization

Optical microscopy and spectroscopy techniques have the versatility and advantage of enabling measurements in various environments such as in solution, *in vitro*, and *in vivo*.¹³ Due to the chirality-dependent fluorescence emission from the SWCNTs, spectrally

resolved optical imaging of the SWCNTs is highly advantageous and critical for thoroughly investigating these nanomaterials, for both fundamental research and biomedical applications. Imaging modalities designed for observing the fluorescence signal from the SWCNTs in complex material and biological systems generally aim to detect either the intensity or the emission spectrum of specific SWCNT chiralities. Hyperspectral imaging is an optical technique that combines spectroscopy and microscopy to spatially resolve the intensity and wavelength information at the same time.¹⁶ The intensity data can be acquired in a similar way to conventional optical microscopes by illuminating the microscope field-of-view with an excitation source and detecting the emission intensities of individual pixels. A broadband (full spectrum) intensity image is composed of photons at different wavelengths and can conceptually be considered as a set of images at each wavelength (Figure 1. 6a).^{13, 16} Additionally, the hyperspectral data can be obtained by sequentially collecting a stack of monochromatic images at each wavelength band, in a wavelength range (Figure 1. 6b). The end result is called a “hyperspectral cube”.¹⁶

The core technology in designing NIR hyperspectral microscopes is a continuously tunable filter based on volume Bragg gratings (VBG), which is rotated with respect to the incoming full-spectrum image to optically isolate each wavelength (Figure 1. 6c and d). To construct a NIR hyperspectral microscope, the VBG is incorporated between the emission port of a standard inverted fluorescence microscope and a NIR camera (Figure 1. 6c and d). By specifying the angle (θ) of a turret-mounted grating with respect to the collimated emission beam from the sample (λ_{All}), a ray of center wavelength ($\lambda_B = 2n\Lambda\sin(\theta)$) is reflected by the grating into the detector, where n is the refractive index and Λ is the period of the grating (Figure 1. 6c). To reduce the bandwidth, the emission can be passed twice

through the VBG. The integration time for each monochromatic image generally ranges from 0.05 – 4 s, depending on the signal; hence, the hyperspectral cube acquisition times range between 20 s and 10 min.¹⁶

Figure 1. 6. Hyperspectral Fluorescence Microscopy. (a) A broadband (full spectrum) image is conceptually composed of a set of images, each at a specific wavelength.¹³ (b) A hyperspectral cube is experimentally constructed by sequentially acquiring a stack of monochromatic images at each discrete wavelength.¹³ (c) Schematic of the VBG demonstrating the refraction of the polychromatic light by the grating to produce a monochromatic beam.¹³ (d) A Reconstruction of the hyperspectral imaging microscope.¹⁶

Although NIR hyperspectral microscopy has the ability to collect an entire set of spectral parameters from SWCNTs with single-pixel resolution in complex biological

systems, it cannot be utilized when fast kinetics data acquisition is required. To enable fast time-resolved experiments, a portable probe-based spectroscopy instrument can be employed for acquiring full spectra from bulk SWCNT samples, such as solutions and composite materials, with time resolutions as low as 0.01 s (Figure 1. 7).¹³ Utilizing a bifurcated fiber optic reflection probe bundle, both excitation and emission lights can be delivered to and captured from the sample through one common end. The advantage of a probe is the versatility of its applications, where it can be aimed at any SWCNT-containing region of a sample or even an animal to detect real-time fluorescence spectra.

Figure 1. 7. Schematic of a novel probe fluorescence spectrometer utilized to study fast kinetics phenomena in bulk materials and animals.¹³

1.5. Novel Applications of the Semiconducting SWCNTs

1. 5. 1. Novel Electronic and Material Applications of the Semiconducting SWCNTs

Due to their electronic bandgap characteristics, the SWCNTs have indicated great potential for electronic, quantum-photonic, and biomedical applications. SWCNTs have been used in molecular electronics devices such as field emitting transistors (FETs) since

they are able to carry enormous current densities (greater than 10^{13} A/m²).⁴² With revolutionary advances in nanofabrication processes, high-performance top-gated carbon nanotube FETs (CNT FETs) with a gate length of 5 nanometers have been fabricated that perform better than silicon complementary metal-oxide semiconductor (CMOS) FETs at the same scale.⁴³ The 5-nanometer CNT FETs have approached the quantum limit of FETs by using only one electron per switching operation. Utilizing a combination of SWCNT processing methods and circuit design techniques, a complete computer has been developed entirely using CNT FETs, capable of running a basic operating system that can implement multiple programs concurrently.⁴⁴ Additionally, a beyond-silicon microprocessor has recently been fabricated entirely from CNT FETs by achieving perfect atomic-level control of variabilities and defects in SWCNTs.⁴⁵

The SWCNTs also exhibit flame-retardant properties,¹⁰ extraordinarily high Young's modulus,⁹ and thermal conductivity⁴⁶; hence, they can act as superior materials if assembled into bulk platforms or ideal fillers that can improve both flammability, and mechanical properties of polymer nanocomposites.

1. 5. 2. Novel Biomedical Applications of the Semiconducting SWCNTs

As mentioned in the previous sections, due to their environmentally sensitive and photostable NIR-II fluorescence, the semiconducting SWCNTs have recently attracted substantial interest for biosensing and bioimaging applications. Moreover, the recent toxicity studies on non-covalently polymer-wrapped SWCNTs have demonstrated short- and long-term biocompatibility *in vivo*.⁴⁷ These results indicate that SWCNTs wrapped in biocompatible polymers and administered with low concentrations (1 µg/mL) do not cause

any noticeable signs of toxicities in preclinical studies, at time points less than four months.⁴⁷ Hybrids of SWCNTs and synthetic or natural polymers have been utilized to design biomolecule detection systems in a wide range of platforms. For instance, Heller *et al.* have engineered prototype optical sensors composed of ssDNA-SWCNTs conjugated to antibodies that respond to various cancer biomarkers.⁴⁸⁻⁵¹ The sensors have been loaded into semipermeable membranes and hydrogels to enable real-time optical detection within the live animals.^{50, 51} The polymer-wrapped SWCNTs have also been extensively investigated by Heller *et al.* and Strano *et al.* to design intracellular optical probes for quantitatively monitoring the single-organelle concentrations of biological molecules in live cells.^{34, 40, 52} The versatility of creating such optical probes along with the advances in microscopy and instrumentation has made them ideal candidates as research tools to study the kinetics of fundamental biological processes in a laboratory setup. In addition to biological detection, the application of the functionalized SWCNTs as imaging agents in neurosciences and cancer diagnostics has been very promising. Landry *et al.* have reported on/off SWCNT probes that enable imaging of synaptic and extrasynaptic catecholamines and their release dynamics in the extracellular space (ECS) of brain tissue.³⁹ Additionally, Cognet *et al.* have developed single-particle imaging platforms to spatially resolve the viscosity of the brain extracellular matrix in acute brain slices based on the single-particle tracking of nanotube trajectories while they diffuse in the ECS upon injection.⁵³ For cancer diagnostics applications, Belcher *et al.* have developed injectable probes composed of SWCNTs coupled to an M13 bacteriophage carrying a modified peptide binding to an extracellular protein overexpressed in ovarian cancer tumors.⁵⁴ Their imaging system can display real-time video imagery to guide intraoperative tumor debulking and postoperative

tumor imaging based on the NIR-II emission from the SWCNTs.

As the number of these exciting technologies based on non-covalent functionalization of the SWCNTs is substantially increasing, imperative to their adoption as standard optical probes and biosensors is to thoroughly understand their physicochemical characteristics. It has been theoretically suggested that these characteristics are mainly a function of the SWCNT chirality and polymer chemical structure.³⁷ As the polymer-chirality pairs are often multiplexed by synthesizing libraries of polymers and hybridizing them with various SWCNT chiralities, utilizing high throughput spectroscopic and microscopic assays is necessary to study their fundamental properties.

Furthermore, although extensively utilized as solution phase biosensors, integrating the functionalized SWCNTs into bulk biomaterial and thin-film platforms provides further avenues for designing a wide range of point-of-care diagnostics devices for non-invasive monitoring of biochemical information. Incorporating the SWCNTs into these platforms has three significant challenges. First, their NIR fluorescence is remarkably sensitive to the chemistry of their local environment and can be suppressed by other components in the biomaterial preparation processes. Second, due to the hydrophilicity of these sensors, it is unfavorable to engage them in any processes involving organic solvents as they form bulk aggregates in hydrophobic environments. Third, utilizing bulk quantities of the polymers and SWCNTs should be avoided to minimize the cost per device. Therefore, novel microfabrication processes should be developed to overcome all these challenges.

The motivation for the presented research arises from these needs and numerous material and biomedical applications of the polymer-wrapped SWCNTs.

1. 6. Thesis Outline

This dissertation takes ssDNA as the most substantial example for SWCNT functionalization and studies the fundamental physicochemical characteristics of ssDNA-SWCNTs, as a function of the ssDNA sequence and SWCNT chirality, by developing various spectroscopy, microscopy, and material characterization assays. The methods and procedures developed in this dissertation can certainly be applied to any other amphiphilic polymer utilized to non-covalently functionalize the SWCNTs and other fluorescent nanoparticles. Following this detailed characterization, the acquired fundamental knowledge will be employed to design, for the first time, flexible biocompatible biosensing devices for optical wearable biosensing based on SWCNTs, utilizing a robust microfabrication process. Below is an outline of the presented material:

Chapter 2

Here, we discuss a combined experimental and analysis platform based on time-resolved NIR fluorescence spectroscopy to extract a complete set of fluorescence parameters, namely excitation wavelength, emission wavelength, emission intensity, and full width at half maximum, to characterize the complexation affinity and stability of ssDNA-SWCNT hybrids, as a function of ssDNA sequence and SWCNT chirality. We also develop an analytical method based on empirical data to identify ssDNA recognition sequences for SWCNT chirality purification.

Chapter 3

Here, we expand a detailed understanding on the cellular stability and retention of the ssDNA-SWCNTs as a function of the ssDNA sequence, utilizing a set of imaging and

spectroscopy techniques. We study the optical stability of the ssDNA-SWCNTs in both wavelength and intensity space utilizing hyperspectral fluorescence microscopy. We then develop a visible microscopy procedure to monitor their intracellular hybrid stability. To correlate the ssDNA-SWCNT optical and hybrid stability to their intracellular fate, we utilize confocal Raman microscopy to accurately quantify their intracellular concentrations over time and perform a pharmacological inhibition study to identify the mechanism of SWCNT expulsion from the cells.

Chapter 4

In this chapter, we call upon an important shortcoming in accurately quantifying the length of the SWCNTs and the ssDNA coverage density on individual nanotubes. We identify an artifact in the process for SWCNT length quantification using AFM and develop a standard assay for AFM-based size quantification.

Chapter 5

In this chapter, we discuss a novel characteristic of the ssDNA-SWCNTs discovered by our group for the first time. We report that the thermal stability of the SWCNTs and other carbon nanomaterials can significantly be enhanced upon non-covalent functionalization with ssDNA. SWCNTs with enhanced thermal stability will be advantageous in numerous high-temperature applications such as high temperature sensing and electronics.

Chapter 6

In this chapter, for the first time, we present a one-step microfabrication process to

produce wearable microfibrinous textiles incorporating functionalized SWCNTs. We thoroughly optimize the fabrication process to obtain a homogeneous SWCNT distribution inside of the individual microfibers. Utilizing confocal Raman microscopy, we characterize the long-term integrity and retention ability of the microfibers. Finally, as the first generation of the SWCNT-based wearable optical biosensors, we propose utilizing this platform to develop a smart wound dressing to spatially and temporally monitor reactive oxygen species concentrations in real-time in the wound environment.

Chapter 7

In this chapter, we summarize our findings, present concluding remarks and propose future directions.

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Chapter 2

DNA-Carbon Nanotube Complexation Affinity and Photoluminescence Modulation are Independent

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2. 1. Summary

Short single-stranded DNA (ssDNA) has emerged as the natural polymer of choice for non-covalently functionalizing photoluminescent single-walled carbon nanotubes. In addition, specific empirically identified DNA sequences can be used to separate single species (chiralities) of nanotubes with exceptionally high purity. Currently, only limited general principles exist for designing DNA-nanotube hybrids amenable to separation processes, due in part to an incomplete understanding of the fundamental interactions between a DNA sequence and a specific nanotube structure, while even less is known in the design of nanotube-based sensors with determined optical properties. We therefore developed a combined experimental and analysis platform, based on time-resolved near-infrared fluorescence spectroscopy, to extract the complete set of photoluminescence parameters that characterize DNA-nanotube hybrids. Here, we systematically investigated the affinity of the $d(GT)_n$ oligonucleotide family for structurally-defined carbon nanotubes by measuring photoluminescence response of the nanotube upon oligonucleotide displacement. We found, surprisingly, that the rate of displacement of oligonucleotides is independent of the coverage on the nanotube, as inferred through intrinsic optical properties of the hybrid. The kinetics of intensity modulation are essentially single exponentials, and the time constants, which quantify the stability of DNA binding, span an order of magnitude. Surprisingly, these time constants do not depend on the intrinsic optical parameters within the hybrids, suggesting that DNA-nanotube stability is not due to increased nanotube surface coverage by DNA. Further, a principal component analysis of the excitation and emission shifts, along with intensity enhancement at equilibrium accurately identified the (8,6) nanotube as the partner chirality to $(GT)_6$ ssDNA. Combined, the chirality-resolved equilibrium and

kinetics data can guide the development of DNA-nanotube pairs with tunable stability and optical modulation. Additionally, this high-throughput optical platform could function as a primary screen for mapping the DNA-chirality recognition phase space.

2. 2. Introduction

The bandgap near-infrared (NIR) photoluminescence from semiconducting single-walled carbon nanotubes (SWCNT) is photostable, tunable, and sensitive to the local environment.¹ Over the last 15 years, significant progress has been made in applying carbon nanotube photoluminescence towards a range of *in vitro* and *in vivo* biomedical applications.² Efforts to synthesize nanotubes of a specific structure (chirality),³ along with methods to separate specific nanotube chiralities have achieved notable successes.⁴

For both biological applications and separation procedures, short single-stranded DNA plays an important role. Pristine single-walled carbon nanotubes require non-covalent functionalization to enable aqueous suspension while still retaining their intrinsic NIR optical properties.¹ Additionally, separation or sensing applications that require a specific biomolecular interaction on the nanotube surface depend on the functionalizing polymer to impart a recognition ability.¹ Short single-stranded DNA was initially identified as a polymer that could efficiently solubilize single-walled carbon nanotubes via non-covalent interactions forming DNA-nanotube hybrids.⁵ Subsequently, reports have shown that DNA facilitates separation of nanotube chiralities by imparting distinct hydrophobicities and/or charge densities on nanotubes by the specific interactions between certain sequences of DNA and specific (n,m) nanotube species.^{6, 7} Additionally, this DNA sequence-nanotube chirality matching, hypothesized to be the result of extended hydrogen bonding networks

amongst adsorbed nucleobases,⁶ inhibits oxygen induced quenching of the nanotube photoluminescence.⁸ Similarly, screening DNA-chirality pairs for analyte specificity⁹ led to the development of optical biosensors for a range of small molecule analytes.¹⁰ The ability to integrate molecular recognition into DNA-nanotube hybrids has been achieved via base-pair hybridization,¹¹ antibody-coupling,¹² and oligonucleotide aptamers.¹³

Several techniques have been employed to probe the fundamental interactions between DNA and the carbon nanotube surface. Absorption spectroscopy assays¹⁴⁻¹⁶ can be used to extract thermodynamic parameters, but are limited in their ability to fully resolve multiple chiralities. Modalities such as atomic force microscopy¹⁷ and thermogravimetric analysis are quantitative,¹⁸ but also cannot resolve nanotube chiralities. Techniques, including single molecule force spectroscopy to directly measure the force required to separate ssDNA from a nanotube¹⁹ and nanoparticle fluorescence spectroscopy to observe DNA-nanotube interactions in a microfluidic cell,²⁰ continue to further our understanding. Molecular dynamics simulations can be used to visualize the DNA structure on a nanotube with unprecedented resolution,²¹ but simulations are costly in time and limited to simple model systems.²² In summary, the techniques currently available are either unable to resolve a sufficient number of individual chiralities or cannot be scaled to represent ensemble averaged data. Therefore, our understanding of the DNA-nanotube interactions at their interface and the resulting optical signal transduction remains incomplete.

The optical transition energies of carbon nanotubes are modulated by the local environment,²³ with the local dielectric constant²⁴ influencing both quantum yields and solvatochromic shifts.²⁵ For DNA-carbon nanotube hybrids, the microenvironment of the nanotube is a complex equilibrium structure that depends on DNA sequence,²⁶ water density,²⁷

and nanotube chirality.²² A recent technique to study the stability of DNA-nanotubes by competing ssDNA with a surfactant²⁰ highlighted the potential of time-resolved spectroscopy to directly measure both optical transition energies and the kinetics of DNA desorption. While these assays were limited to physically-separated single nanotube chiralities, optical advancements such as real time hyperspectroscopy²⁸ allow for spectral separation of individual chiralities in an unsorted sample. By directly measuring equilibrium optical transitions and transient intensity modulations, we could address unanswered questions in the literature, such as: Is DNA stability a function of DNA sequence length, or nanotube chirality, or both? Does the degree of nanotube surface coverage by DNA determine the stability of the DNA-nanotube hybrid, and how does this answer depend on the nanotube chirality? Can optical signatures help screen and identify target chiralities for separation by DNA sequences?

Here, we examined DNA-nanotubes for their binding affinities and optical modulations, using a combination of photoluminescence excitation/emission (PLE) plots and time-resolved near-infrared photoluminescence spectroscopy. We found that stability of DNA on a nanotube could not be predicted from equilibrium photoluminescence modulations, but required a kinetics measurement *via* a surfactant induced DNA displacement. The kinetics of DNA displacement were single exponential, with time constants that spanned an order of magnitude. Interestingly, these time constants were essentially independent of nanotube surface coverage, as inferred by relative excitation/emission energies, suggesting that the strength of DNA binding is the primary determinant of stability of the DNA-nanotube hybrid. We propose that our high throughput spectroscopy and analysis

method can screen and identify DNA-chirality pairs which optimize photoluminescence response and stability for both sensing and separation applications.

2. 3. Results and Discussion

To systematically investigate DNA-nanotube interactions as a function of nanotube chirality and DNA-sequence length, we prepared ss(GT)_n-nanotube hybrids where n=3, 6, 9, 12, 15, or 30 repeats. As apparent from the high peak-to-valley ratios and narrow bands in the absorption spectra (Figure S2. 1), regardless of DNA sequence length, the entire mixture of HiPco nanotubes were well dispersed. For the equilibrium conformation of ss(GT)_n-nanotubes (Figure 2. 1a), we obtained the intensity, excitation, and emission peaks for the (8,3), (7,5), (10,2), (9,4), (7,6) and (8,6) chiralities, from the initial photoluminescence excitation/emission (PLE) plots (Figure 2. 1b).

Figure 2. 1. Schematic of the surfactant-induced DNA displacement mechanism and corresponding optical output. (a) Equilibrium conformation of DNA on a nanotube, and the (b) corresponding optical parameters. (c) Addition of SDC changes the emission spectra (d) in time. (e) The equilibrium SDC-SWCNT and the (f) corresponding optical parameters.

We then added excess sodium deoxycholate (SDC), known to displace the DNA from the nanotube surface.²⁹ On adding SDC (0.1% final concentration), we observed the

dynamic process of surfactant binding to the exposed nanotube surface (Figure 2. 1c) and the resultant modulation in the nanotube emission spectra (Figure 2. 1d) upon excitation at either 660 nm or 730 nm (red arrows in Figure 2. 1a). At equilibrium, the DNA initially adsorbed on each nanotube chirality is completely displaced by SDC (Figure 2. 1e), demonstrated by the convergence of all optical transition wavelengths (Figure S2. 2) obtained from the final PLE plots (Figure 2. 1f). When compared with the excitation and emission peaks from SDC-nanotubes (i.e. SWCNT dispersed directly in SDC), we find that the DNA-nanotube spectra on SDC addition are as blue-shifted or more blue-shifted than the corresponding SDC-nanotube spectra. This discrepancy is likely due to the differential ability of SDC and DNA to singly exfoliate the nanotube samples.³⁰ The resulting data of photoluminescence modulation of DNA-to-SDC replacement on the nanotube surface are presented in Table S2. 1 and S2. 2.

The evolving emission spectra (Figure 2. 1d) is an ensemble average of nanotubes in solution, with each nanotube containing sections with either DNA or SDC coverage. When fit with a Lorentzian profile,³¹ we extracted peak intensity (Figure 2. 2a), center wavelength (Figure 2. 2b) and full-width-at-half-maximum (FWHM, Figure 2. 2c) as a function of time, for each of the 36 sequence-chirality pairs (Figure S2. 3 and S2. 4). Interestingly, certain DNA-chirality pairs (e.g. (GT)₆-(10,2)) exhibit a large initial blue-shift followed by slow red-shift to an equilibrium value. We treated each emission spectrum (Figure S2. 5a) as a linear combination of the DNA-only and SDC-only spectrum²⁹ (Figure S2. 5b), and fit all transient spectra (Figure S2. 5c) as the sum of these two components (Figure S2. 5d).

Figure 2. 2. Modulation of emission parameters as a function of time. (a) Intensity, (b) center wavelength and (c) full-width at half-maximum of the (GT)₃₀-(8,6) hybrid following SDC addition.

The anti-correlated contributions of the DNA and SDC components, plotted as a function of time (Figure S2. 5e), suggest a concomitant decrease in DNA coverage and increase in SDC coverage. However, as the blue-shifted SDC-peak is significantly (~ 4 -fold) brighter than the DNA peak, the apparent wavelength shift is faster than the intensity increase (Figure S2. 5f). We observed this phenomenon in the experimental data for every sequence-chirality pair (Figure S2. 3 and S2. 4). For the rest of our analysis, we used the peak intensity at the SDC-emission wavelength as a metric for the degree of DNA displacement due to replacement by SDC.

As a relative measure of the DNA-nanotube hybrid stability, we plotted the emission intensity of the SDC-peak wavelength as a function of time, for each DNA sequence-nanotube chirality pair. The majority (30/36) time traces of the fractional increase in intensity (Figure 2. 3) were fit with a single exponential association (Table S2. 3, $r^2 > 0.95$ for all time traces). However, traces for (8,3) and for the shorter sequences (6/36 in total) were significantly better fit as a sum of two exponentials, suggesting a more complicated two-stage mechanism for these DNA-chirality pairs (Table S2. 4). Dashed lines in Figure S2. 3 represent the best fits (single or double exponential) for each sequence-chirality pair.

Physically, the processes occurring on the nanotube surface include (1) binding of SDC to the exposed nanotube surface, (2) rearrangement and displacement of DNA by SDC, and (3) SDC reorganization and micelle formation over the nanotube surface.

Figure 2. 3. Fractional intensity increase (of the SDC peak) as a function of time for all (GT)*n* sequences for the (a) (8,3), (b) (7,5), (c) (10,2), (d) (9,4), (e) (7,6) and the (f) (8,6) chirality. Dotted lines indicate the exponential fits for each sample.

We propose that the separate rates of the above processes might be too fast to experimentally observe (and at SDC concentrations above 0.1 %, even the single exponential behavior was too fast to fit within the experimental time resolution of 0.5 s). Future studies, including titrating the SDC concentration to develop a Langmuir adsorption model, or performing the kinetics measurements at lower temperatures or lower SDC concentrations, could provide further insight into the mechanisms being investigated.

For comparing across samples, we used the time constants from single exponential fits as an experimentally derived metric for DNA-nanotube hybridization affinity. The combined kinetics and PLE data provide ten descriptors of each sequence-chirality pair

that can be used to assess stability and dynamic range in optical responses, respectively, in DNA-nanotube hybrids.

We asked if photoluminescence parameters and their modulations could predict sequence-chirality recognition pairs and potentially enable optical screening to identify target chiralities for specific DNA sequences. We thus assessed excitation shifts, emission shifts, and intensity enhancements for all DNA-chirality pairs and compared visually using heat maps (Figure 2. 4a-c). Certain trends were visible in the data, such as the large excitation shift and intensity enhancement for (8,6), and the maximum emission shift for (9,4). We systematically looked for correlations between the five unique photophysical parameters, both within individual chiralities and across the entire nanotube population. As all sequence-chirality pairs converged to the same SDC-coated nanotube sample (Figure S2. 2), the initial excitation and emission peaks shifted to the same value. To our surprise, intensity enhancement was not correlated to either excitation or emission shift for any chirality, and only the (8,6) showed a statistically significant dependence between the excitation and emission energy shifts (Figure S2. 6). However, excitation energy shifts and emission energy shifts were weakly correlated with intensity enhancement at the population level (0.369 and 0.395 Spearman correlation respectively, $p < 0.05$ for both), suggesting a potential connection between the DNA sequence family and the nanotube chirality.

To investigate the sequence-chirality connection to these optical parameters, we used principal component analysis on the excitation shift, emission shift, and intensity enhancement data for the DNA sequence-nanotube chirality pairs. From the three principal components, over 87% of the variance in the data could be explained by the first two. A scatter plot of the first two principal components maximizes the variance in the data while

reducing the dimensionality from three to two. We asked whether the DNA sequence-nanotube chirality pairs in the principal component phase space clustered in a meaningful way. A two-dimensional k-means algorithm (without any initial guesses) identified four distinct clusters (Figure 2. 4d).³²

Figure 2. 4. Heat map, as a function of nanotube chirality and DNA sequence, of the (a) excitation energy shifts, (b) emission energy shifts and the (c) fractional intensity increases. The nanotube chiralities are arranged in order of decreasing bandgap energy. (d) Principal component analysis of the excitation shift, emission shift, and intensity enhancement, for the first two principal components.

Interestingly, one cluster (labeled 4 in Figure 2. 4d) was composed entirely of all six ss(GT)_n-(8,6) pairs (Table S2. 5). Though no optical parameter exclusively selects the (8,6) chirality, k-means analysis of the principal components differentiates the optical response of all (8,6) chirality nanotubes from the other 30 DNA sequence-nanotube chirality

pairs. As ss(GT)₆ was initially identified as the recognition sequence for the (8,6) chirality using ion-exchange chromatography,⁶ the identification of (GT)_n-(8,6) nanotubes as a distinct cluster in principal component analysis is a promising result. Though photoluminescence measurements of the addition of small molecules to polymer-nanotube hybrids have highlighted the complexity of the interactions,^{33,34} the data suggests that our method, validated for one specific DNA-sequence family and chirality, could potentially provide a scalable and predictive approach to determining candidate DNA recognition sequences for specific nanotube chiralities.

To characterize DNA-nanotube hybridization affinities, we generated a heat map of the time constants as a function of nanotube chirality and DNA sequence length (Figure 2. 5a). Visually apparent, the heat map indicated a general trend, with longer time constants more prevalent amongst DNA-nanotube hybrids with longer DNA and larger diameter nanotubes. When mapped in ascending order, the time constants (larger time constant indicating a stronger binding affinity of DNA to the nanotube) increased from 5 to 80 seconds (Figure 2. 5b). A one-dimensional k-means analysis identified three classes of samples – least stable (time constants < 25 s), stable (25 s < time constants < 50 s) and a small subset that are exceptionally stable (time constant > 50 s). Interestingly, ss(GT)₁₂-(8,6) and ss(GT)₆-(8,6) stand out as outliers, with high DNA-nanotube affinity despite the comparatively short DNA sequence length.

Figure 2. 5. DNA stability as a function of DNA-SWCNT parameters (a) Heat map of single exponential time constant of fractional intensity increase as a function of DNA sequence length and chirality. (b) Time constants plotted in ascending order and color-coded by chirality. (c) Mean time constant (gray squares) as a function of DNA sequence length, with data points for each chirality represented as color-coded circles. (d) Mean time constant (gray squares) as a function of chirality, with data points for each DNA sequence represented as color-coded circles. Error bars are the s.e.m. across the contributing data points.

Next, we analyzed the time constants as a function of either sequence length or chirality. For the combined nanotube population, the stability of the ss(GT)_n DNA correlated strongly with sequence length (Figure 2. 5c). However, the chirality-resolved data indicated this relationship to only be true for three out of six chiralities (Figure S2. 7). The time constants for each DNA sequence length did not correlate with nanotube chirality either (Figure 2. 5d), and the range of time constants across the six chiralities was large.

Based on these results, we propose that DNA-nanotube binding affinity is strongly dependent on the specific interaction between a nanotube chirality and the DNA sequence, with no obvious systematic dependence on either. In the most striking example, ss(GT)₃-(9,4) and ss(GT)₃₀-(7,5) had essentially identical time constants (12.48 s vs 14.63 s, respectively), despite a 54-base sequence length advantage for ss(GT)₃₀-(7,5). Even within a single chirality, such as the (8,3), ss(GT)₃-(8,3) and ss(GT)₃₀-(8,3) had statistically identical time constants (~17.5 s). This analysis suggests that increased hybrid stability is not solely determined by the length of DNA sequence.

As the displacement of DNA by SDC on the nanotube surface induces a change in the NIR photoluminescence, we asked whether photoluminescence parameters correlated with the stability directly measured via DNA displacement kinetics. We hypothesized that the stability for a specific sequence-chirality pair could result from the extent of nanotube surface coverage by DNA. A nanotube with low DNA coverage would provide a larger and more accessible surface for oxygen and water molecules, when compared to a nanotube with high DNA coverage (Figure 2. 6a). Because water increases the local dielectric constant around the nanotube,²³⁻²⁵ a more stable DNA sequence-nanotube chirality pair with low surface exposure to water (high DNA coverage) should have excitation and emission peaks that correspond to higher energy optical transitions.^{26, 27} Instead, scatter plots of the excitation and emission energy peaks showed no correlation with the time constants, for any of the six chiralities (Figure 2. 6b-c). The intensity enhancement, often used as a proxy for nanotube surface coverage,^{10, 20} also did not correlate with the time constants (Figure 2. 6d). No correlation was observed between photoluminescence parameters and DNA sequence length either (Figure S2. 8). As DNA stability on the nanotube does not appear to

correspond to the relative excitation and emission peak positions, we conclude that DNA surface coverage is not the determining factor for DNA-nanotube hybridization stability. We propose that the strong binding observed between certain DNA sequences and nanotube chiralities is likely due to specific DNA conformations (Table 1), which has been suggested to enhance hydrogen bonding between DNA bases.^{6, 22}

Figure 2. 6. (a) Schematic for a DNA-nanotube with low DNA coverage and high DNA coverage. (b) Initial excitation peak, (c) initial emission peak and (d) intensity enhancement as a function of time constant for all DNA-nanotube samples, segmented by chirality.

Table 2. 1. Correlations between photoluminescence parameters and sequence length. Correlations presented as (Spearman Correlation, significance). Values presented are Spearman correlation coefficient and significance.

Parameters Compared	Nanotube Chirality						Combined
	(8,3)	(7,5)	(10,2)	(9,4)	(7,6)	(8,6)	
Sequence Length vs I/I_0	-	-	-	-	-	-	-
Sequence Length vs Initial Excitation	-	-	-	0.83, 0.040	-	-	-
Sequence Length vs Initial Emission	-	0.94, 0.0048	-	-	-	-	-
Time Constant vs Sequence Length	-	-	0.83, 0.042	0.89, 0.019	1, 0	-	0.53, 0
Time Constant vs Intensity Enhancement	-	-	-	-	-	-	-
Time Constant vs Initial Excitation	-	-	-	-	-	-	-0.41, 0.02
Time Constant vs Initial Emission	-	-	-	-	-	-	-0.51, 0.013
Intensity Enhancement vs Excitation Shift	-	-	-	-	-	-	0.37, 0.027
Intensity Enhancement vs Emission Shift	-	-	-	-	-	-	0.39, 0.017
Excitation Shift vs Emission Shift	-	-	-	-	-	-0.94, 0.048	-
Initial Excitation vs Initial Emission	-	-	-	-	-	-	-

2. 4. Conclusions

We have developed a framework for experimentally determining both the stability and optical modulation of DNA-nanotube hybrids. By all indications, DNA-nanotube stabilities and photoluminescence modulations are essentially independent (Figure 2. 7). In the assay presented, equilibrium and dynamic measurements of DNA displacement from the nanotube surface by a surfactant allow both properties to be observed simultaneously. The DNA displacement follows single exponential kinetics, with time constants that range over an order of magnitude. Surprisingly, the stability appears to depend on the molecular interactions between a DNA sequence and a specific nanotube chirality, with no systematic

dependence on DNA sequence length or chirality. Optical parameters that reflect accessibility of the solution to the nanotube surface do not correlate with the stability of the DNA-nanotube hybrid, suggesting that stability does not result from an increase in DNA coverage of the nanotube surface. However, principal components analysis and k-means clustering of optical parameters can identify the nanotube chirality with an enhanced affinity for a DNA family. As a result, predictions that are tailored for specific biological applications can be made. For instance, a low-stability but high-dynamic response DNA-nanotube pair like the ss(GT)₃-(9,4) hybrid can be selected for detecting amphiphilic proteins that displace DNA. Alternatively, a high-stability, high-dynamic response like the ss(GT)₆-(8,6) pair may be suitable for long-term monitoring of analytes in biological samples. We envision our optical screening approach enhancing the study of fundamental DNA-nanotube interactions, and in the rational design of DNA-nanotube sensors for a variety of biological applications. The ability to optically identify recognition sequences for specific nanotube chiralities is another potentially valuable application.

Figure 2. 7. Model for photoluminescence parameters and hybridization affinities in DNA-nanotube hybrids.

2. 5. Methods

Nanotube Sample Preparation: HiPco single walled carbon nanotubes (Unidym, HiPco Raw) were suspended with DNA in 1 mL of deionized water with 100 mM NaCl (Sigma-Aldrich) by adding 1 mg raw nanotubes to 2 mg of desalted ss(GT)_n (n=3,6,9,12,15, or 30) oligonucleotide (Integrated DNA Technologies) in a microcentrifuge tube. The mixtures were ultrasonicated using a 1/8" tapered microtip (Sonics & Materials, Sonics Vibracell) for 30 min at 40% amplitude, with an average power output of 8 Watts, in a 0 °C temperature-controlled microcentrifuge tube holder. After sonication, the dispersion was ultracentrifuged (Sorvall Discovery 90SE) for 30 min at 250 000g in a fixed-angle rotor (Fiberlite F50L), and the top 80% of the supernatant was extracted. The concentration was determined with a UV/vis/nIR spectrophotometer (Jasco, Tokyo, Japan) using the extinction coefficient $A_{910} = 0.02554 \text{ L} \cdot \text{mg}^{-1} \cdot \text{cm}^{-1}$.³⁵

Near-Infrared Photoluminescence Spectroscopy: Rapid two-dimensional excitation/emission photoluminescence contour maps were constructed using a procedure outlined in a previous study.³⁶ Briefly, a supercontinuum laser (NKT SuperK Extreme EXR15) coupled to a variable bandpass filter (NKT SuperK Varia High) was used to excite DNA-nanotube sample contained within a 96-well plate. The excitation wavelength varied from 500 to 827 nm with a 20 nm bandwidth and step size of 3 nm. The emissions from the nanotube samples were collected, fed into a spectrometer with an 86 groove/mm ruled grating, 320 mm focal length and f/4.6 aperture ratio (Princeton Instruments IsoPlane SCT 320), and finally directed into a TE-cooled InGaAs array detector (Princeton Instruments 640 X 512 pixel NIRvana: 640) with a 20 μm pixel size, and Q.E. > 85% in the detection range of 0.9 to 1.7 μm .

In a typical experiment, 100 μL of each DNA-nanotube sample were plated into a clear-bottom optical imaging 96-well plate (Corning) at a defined nanotube concentration of 5 mg/L. From stock concentrations, DNA-nanotube samples were diluted in 100 mM NaCl or 100 mM NaCl + 0.1% sodium deoxycholate (SDC). The well plate was covered and incubated for 12 hours at room temperature in order to ensure an equilibrium in the SDC containing wells. Two-dimensional excitation/emission spectroscopy was performed on all the wells with an exposure time of 0.25 or 1 second at each excitation wavelength for samples with and without SDC, respectively. All samples were examined in triplicate. Custom Labview and MATLAB codes were written to automate the data acquisition and processing, respectively.

For experiments involving the real-time addition of SDC to DNA-nanotubes, the excitation wavelength was set to either 660 or 730 nm, with a bandwidth of 20 nm. The emission spectra were collected in rapid succession with a constant exposure time of 0.25 seconds. After an initial time period of ~ 10 seconds to collect ample DNA-nanotube control data, 1 μL of a stock solution of 10% SDC was spiked into the well and immediately mixed to give a final concentration of 0.1% SDC. All SDC addition experiments were performed in triplicate.

Statistical Analysis: Statistical measures and tests for correlations, in addition to exponential fitting were performed in OriginPro 8.6. PCA and k-means clustering were performed using the in-built functions in Matlab 2014a.

2. 6. Acknowledgments

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Chapter 3

Biomolecular Functionalization of a Nanomaterial to Control Stability and Retention within Live Cells

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3. 1. Summary

Non-covalent hybrids of single-stranded DNA and single-walled carbon nanotubes (SWCNTs) have demonstrated applications in biomedical imaging and sensing due to their enhanced biocompatibility and photostable, environmentally-responsive near-infrared (NIR) fluorescence. The fundamental properties of such DNA-SWCNTs have been studied to determine the correlative relationships between oligonucleotide sequence and length, SWCNT species, and the physical attributes of the resultant hybrids. However, intracellular environments introduce harsh conditions that can change the physical identities of the hybrid nanomaterials, thus altering their intrinsic optical properties. Here, through visible and NIR fluorescence imaging in addition to confocal Raman microscopy, we show that the oligonucleotide length controls the relative uptake, intracellular optical stability, and retention of DNA-SWCNTs in mammalian cells. While the absolute NIR fluorescence intensity of DNA-SWCNTs in murine macrophages increases with increasing oligonucleotide length (from 12 to 60 nucleotides), we found that shorter oligonucleotide DNA-SWCNTs undergo a greater magnitude of spectral shift and are more rapidly internalized and expelled from the cell after 24 hours. Furthermore, by labeling the DNA with a fluorophore that dequenches upon removal from the SWCNT surface, we found that shorter oligonucleotide strands are displaced from the SWCNT within the cell, altering the physical identity and changing the fate of the internalized nanomaterial. Finally, through a pharmacological inhibition study, we identified the mechanism of SWCNT expulsion from the cells as lysosomal exocytosis. These findings provide a fundamental understanding of the interactions between SWCNTs and live cells as well as evidence suggesting the ability to control the biological fate of the nanomaterials merely by varying the type of DNA

wrapping.

3. 2. Introduction

Single-walled carbon nanotubes (SWCNTs) have attracted substantial attention in the nanotechnology field due to their unique set of electrical,¹ physical,² and optical properties.³ Their electronic band gap energies are dependent on their chiral identity, denoted by integers (n,m) , and vary based on diameter and rollup angle,⁴ resulting in semiconducting species which exhibit band gap photoluminescence.³ Although highly hydrophobic in their raw as-produced form, non-covalent functionalization of SWCNTs using surfactants^{5, 6} or amphiphilic biomolecules⁷⁻⁹ has been shown to effectively disperse SWCNTs into aqueous solutions while preserving their intrinsic optical properties. Single-stranded DNA can non-covalently functionalize SWCNTs via π -stacking of hydrophobic bases onto the SWCNT sidewall, while the hydrophilic phosphate backbone allows for significantly enhanced aqueous solubility.¹⁰ These DNA-SWCNT hybrids have shown promise as biological imaging¹¹ and sensing probes¹² due to their near-infrared (NIR) photoluminescence which is tunable, photostable, and sensitive to their local environment.¹³⁻¹⁶

Hybrids of DNA and SWCNTs are preferred over other non-covalent approaches due to their enhanced biocompatibility,¹⁷ ability to sort single (n,m) -chiralities from parent mixtures,^{18, 19} and the potential for sensing imparted by the inherent diversity of oligonucleotide sequence.²⁰ Specific sequence formulations of DNA-SWCNTs have been recently used to detect miRNA *in vivo*²¹ in addition to reporting lipid concentrations in live cells²² and animals,²³ while other approaches have used similar oligonucleotide surface

modifications for DNA or siRNA delivery both *in vivo*^{24, 25} and in plants for controllable gene regulation.^{26, 27} Although these advances are promising displays of the utility of nanoscale technology, fundamental questions relating the identity of these sensors after prolonged exposure within the biological environment remain largely unexplored. The potential instability of such DNA-SWCNT sensors has direct implications on their ability to perform a designated task, yet the indirect consequence is a nanomaterial with altered properties from its original state. The changed identity of such nanomaterials can cause concerns about toxicity and the unknown effects imparted on the immediate biological environment. While many types of DNA-SWCNTs have been studied extensively *in situ* both computationally²⁸⁻³⁰ and experimentally,³¹⁻³⁶ their direct translation to more complex biological systems cannot be assumed.

Nanomaterials can be designed to enter the body via ingestion, injection, inhalation *etc.*, yet macrophages are typically the first cells to detect and internalize foreign molecules regardless of entry method.³⁷ Macrophages are the immune system's first line of defense, whether as a primary response to a wound or to engulf foreign substances such as nanoparticles that enter the bloodstream.³⁸ Various studies have shown that macrophages internalize DNA-SWCNTs via endocytosis and phagocytosis through the endolysosomal pathway, eventually leading to localization within the lysosomes^{22, 39, 40} and accumulation in the liver macrophages of mice *in vivo*.^{23, 41} Once entrapped within the lysosomes, SWCNTs can remain for days where they experience biologically low pH and exposure to more than 60 hydrolases meant for catabolic degradation.⁴² In these conditions, surface modifications can play a large role on a nanoparticle's ultimate fate, whether degradation, exocytosis, or lysosomal escape.³⁷ Given their extremely high surface area to volume ratio,

small changes in surface functionalization of SWCNTs can make a major impact on their functionality and stability in such environments.

While oligonucleotide length determines the intrinsic stability of the resultant hybrid with a SWCNT in water,³³ little is known how this length of DNA can affect the stability of SWCNTs in complex intracellular environments. Herein, we present an investigation of the physical and optical stabilities of (GT)_n-SWCNTs, where *n* is the number of sequence repeats, upon internalization into murine macrophages. Near-infrared hyperspectral microscopy in live cells revealed strong correlations between oligonucleotide length, NIR fluorescence intensity, and spectral stability of the examined SWCNTs. All DNA-SWCNT combinations displayed emission shifts to lower energies (*i.e.* red-shifts) upon interacting with the cells, however several chiralities of (GT)₆-SWCNTs exhibited significant blue-shifts over the course of 24 hours, indicating molecular adsorption and/or DNA displacement. We quantified SWCNT concentrations in cells using confocal Raman microscopy, which can detect all SWCNTs including non-fluorescent species, and revealed significant differences in both internalization and lysosome-mediated expulsion of (GT)₆- and (GT)₃₀-SWCNTs over 24 hours. Finally, we used fluorophore labeled DNA to probe the condition of the SWCNT hybrids as they were processed through the endolysosomal pathway.

3. 3. Results and Discussion

To study the effects of single-stranded DNA length on the intracellular optical properties of DNA-SWCNTs, we first non-covalently functionalized HiPco SWCNTs with one of five different (GT)_n oligonucleotides, where *n* = 6, 9, 12, 15, or 30 repeats (Figure

S3. 1, Table S3. 1). Murine macrophages (RAW 264.7 cell line) were incubated with 1 mg/L of each (GT)_n-SWCNT sample for 30 minutes under standard cell culture conditions and replenished with fresh media (hereby referred to as a “pulse” of DNA-SWCNTs). The majority of cells exhibited substantial NIR broadband fluorescence (ca. 900-1600 nm) when excited by a 730 nm laser (Figure 3. 1a).

Figure 3. 1. Length-dependent intracellular fluorescence of DNA-SWCNTs. **(a)** NIR fluorescence image of live macrophages pulsed with (GT)₁₅-SWCNTs, along with respective transmitted light image and merged NIR/transmitted light image. Scale bar = 40 μm. **(b)** NIR fluorescence images of macrophages after 30-minute pulse of (GT)_n-SWCNTs, imaged over the course of 24 hours. Scale bar = 20 μm. **(c)** Histograms corresponding to the 0- and 24-hour (GT)_n-SWCNT images in (b), and **(d)** average intracellular fluorescence intensities for all examined DNA sequences 0-, 6-, or 24-hours after (GT)_n-SWCNT pulse. Experiments were performed in triplicate and are represented as mean ± s.d. (* p < 0.05, ** p < 0.01, according to two-tailed two-sample t-test).

In agreement with previous studies,^{15, 22, 43} NIR fluorescence movies confirmed the internalization of the SWCNTs into endosomal vesicles, which were actively translocated around the cell and could be easily distinguished from background cellular autofluorescence (Movies S3. 1 and S3. 2). The NIR fluorescence images were acquired 0, 6, or 24 hours after an initial pulse to assess the DNA length and temporal dependencies on intracellular fluorescence intensity (Figure 3. 1b). In general, the observed NIR fluorescence intensities visibly increased with increasing oligonucleotide length and decreased in time after initial loading into the cells. Histograms constructed from pixel intensity values of the 0- and 24-hour images confirmed that the temporal decreases in intensities were similar amongst all sequences (Figure 3. 1c). Interestingly, the initial intensity distributions were much broader in longer oligonucleotide sequences, suggesting more heterogeneity in the optical response to internalization of these SWCNTs. To quantify the images, the average fluorescence intensities were extracted using a global thresholding analysis to examine the NIR fluorescence from only SWCNTs contained within the cells. We observed significant increases in NIR fluorescence intensities as a function of DNA length (Figure 3. 1d) at each time point. Pearson correlation coefficients (r_p) were determined to be 0.846, 0.885, and 0.850 at 0, 6, and 24 hours respectively, confirming that the correlation was linear and statistically significant ($p < 0.001$ for all) between sequence length and fluorescence intensity at any given time point. To mitigate variations in fluorescence quantum yield (Figure S3. 2), the images from Figure 3. 1b were normalized to each (GT)_n-SWCNT's average 0 hour intensity (Figure S3. 3a) and the percent change in initial intensity was quantified (Figure S3. 3b). While most DNA-SWCNTs demonstrated significant fluorescence quenching in time, (GT)₆-SWCNTs first increased

nearly 25% at 6 hours before decreasing to more than 25% below the initial intensity after 24 hours. Among other factors, it is known that the fluorescence intensity of a SWCNT is inversely correlated to the density of water in the immediate vicinity.⁴⁴ Thus, a removal of water from the surface of (GT)₆-SWCNTs through the adsorption of other amphiphilic molecules can explain the increase in intensity observed at 6 hours. Altogether, we propose that these discrepancies in intracellular fluorescence are affected by (1) variations in DNA-SWCNT interaction with and internalization into cells as a function of DNA length, (2) variations in the optical stability of the (GT)_n-SWCNT hybrids after interacting with and/or internalizing into the cells, or (3) variable rates of cellular expulsion. Throughout the letter, we will carefully examine these hypotheses.

The relationship between stability and fluorescence of DNA-SWCNTs is highly dependent on SWCNT chirality as well as oligonucleotide length.³³ Therefore, we employed NIR hyperspectral fluorescence microscopy to assess the chirality-resolved intracellular stability of the (GT)_n-SWCNTs.¹⁵ Using a 730 nm excitation laser, we were able to resolve four distinct bands in the NIR region corresponding to the emission spectra of the four brightest SWCNT chiralities, (10,2), (9,4), (8,6), and (8,7) (Figure S3. 4a).¹⁵ Hyperspectral images were acquired immediately following a 30-minute pulse of each (GT)_n-SWCNT and after an additional 24 hours of incubation in SWCNT-free cell media (Figure S3. 4b-f). Upon internalization, we observed two common characteristics of all fluorescence spectra: (1) an initial red-shift (*i.e.* increase in wavelength) of every chirality compared to the spectra acquired in cell culture media and (2) increased intensities of longer wavelength chiralities relative to shorter. To explain the first finding, a red-shift in SWCNT emission spectra can be caused by charged species that interact with the phosphate

backbone of DNA and induce a conformational change, ultimately modulating the dielectric environment of the SWCNT and thus shifting SWCNT emission to longer wavelengths.⁴⁵ Surface proteins present on cell membranes with high charge densities have been shown to promote this red-shift upon first contact with DNA-SWCNTs before endocytosis.⁴³ Additionally, the exposure of DNA-SWCNTs to serum-containing cell culture media can produce aggregation that causes spectral modulation by protein-DNA electrostatic interactions.⁴⁶ We attribute the initial red-shift observed to a combination of these factors directly following a pulse of (GT)_n-SWCNTs, in which the macrophages contained both membrane-bound particles that had not yet been internalized as well as newly-formed endosomal vesicles that essentially forced DNA-SWCNTs to form small aggregate complexes with other phagocytosed proteins and cargo. Regarding the second finding, changes in the ratiometric intensities between shorter and longer emission wavelength SWCNTs have been described by inter-nanotube exciton energy transfer (INEET),⁴⁷ a phenomenon that behaves similarly to Förster resonance energy transfer and could be the result of closely packed DNA-SWCNTs contained within lysosomes.

To further compare the NIR fluorescence stabilities across SWCNT chiralities, we converted the emission center wavelengths to energies (meV) and computed the change in emission energy relative to solution controls acquired in cell culture media. Of all the examined oligonucleotide lengths, only the (GT)₆-SWCNTs exhibited significant increases in emission energies in multiple chiralities over 24 hours of intracellular processing (Figure 3. 2a, Table S3. 2), while the other DNA-SWCNTs commonly displayed a moderate loss of energy over the same period of time despite presumably identical intracellular conditions. Previous studies have demonstrated that this increase in emission energy could

arise from endosomal lipids binding to the exposed SWCNT surface,^{22, 23} however the maximum shift in emission energy was not observed at the same time for each chirality, indicating the shifts could be a convolution of multiple physical mechanisms. The influence of a lowered pH to mimic the lysosomal environment was also considered as a potential modulator of SWCNT emission. However, most (GT)_n-SWCNTs exposed to cell media with pH of 4.5 experienced random, small (< 2meV) shifts compared to physiological pH (Figure S3. 5). We believe these results indicate that longer oligonucleotides protect the SWCNT surface from competitive molecular adsorption.

Figure 3. 2. DNA length- and SWCNT chirality-dependent intracellular NIR fluorescence stabilities. (a) Heat maps representing average intracellular change in SWCNT emission energy compared to controls in cell culture media, delineated by chirality, as a function of DNA-sequence and time. All experiments were performed in triplicate. Overlay of transmitted light and hyperspectral images of RAW 264.7 pulsed for 30-minutes with (b) (GT)6- or (c) (GT)30-SWCNTs. Color scale maps to the fitted center emission energy of (9,4)-SWCNTs and histograms represent center emission energies of all SWCNT-containing pixels in each respective image. Bin size = 4 meV. Gaussian functions were fitted to binned data and overlaid with respective R^2 , FWHM, and center emission energies (E_c). Scale bar = 20 μ m.

To assess spectral shifts in SWCNT emission at the single-cell level, we created hyperspectral maps of the shortest and longest oligonucleotide DNA-SWCNTs (*i.e.* (GT)₆ and (GT)₃₀, respectively). By fitting each SWCNT-containing pixel of a hyperspectral image to a Lorentzian curve,^{15, 22} we were able to overlay transmitted light images with center emission energy maps for the (9,4)-SWCNT, *i.e.* the most abundant and brightest SWCNT under 730 nm excitation in HiPco, and construct histograms for each image to depict the intracellular change in SWCNT emission energy change through time (Figure 3. 2b-c). Immediately following a 30-minute pulse (“0 hours”), the average emission energies of (GT)₆-SWCNTs and (GT)₃₀-SWCNTs were statistically identical. Additionally, by fitting the pixel histograms to a Gaussian distribution, the heterogeneity in the populations could be assessed by examining the full width at half maximum (FWHM). In doing so, we uncovered that the FWHM of (GT)₆-SWCNTs immediately after internalization was more than double that of (GT)₃₀-SWCNTs. While the emission energy of (GT)₃₀-SWCNTs showed little change in time, (GT)₆-SWCNTs displayed an 8 meV increase in emission energy and ~50% decrease in FWHM after 6 hours. We believe these DNA-length dependent NIR fluorescence modulations are the result of variations in the relative abundance of oligonucleotide strand ends surrounding each SWCNT. For a given weight of DNA in a DNA-SWCNT hybrid, (GT)₆-SWCNTs have 5 times the number of oligonucleotide strand ends than (GT)₃₀-SWCNTs assuming a similar degree of surface coverage. We propose that these strand ends can act as initiation sites for amphiphilic biomolecules to interact with and adsorb onto the exposed nanotube surfaces, leading to higher overall surface coverages (reduced water densities) and thus greater emission energies.^{22, 44, 48} Consequently, the wide FWHM initially displayed by (GT)₆-SWCNTs was

likely the result of individual SWCNTs responding to the varying local environments through progressing stages of the endosomal pathway, while the reduced FWHM and blue-shift after 6 hours can be attributed to molecular adsorption by lysosomal molecules and rearrangement or displacement of the oligonucleotide wrapping on the majority of SWCNTs. Interestingly, after 24 hours the emission energy slightly decreased closer to its initial value while the FWHM increased towards its initial value, revealing that the hybridized (GT)₆-SWCNTs observed at 6 hours were ultimately unstable.

Due to the large variations in spectral stability of the internalized DNA-SWCNTs, we devised an assay to probe their integrity of the hybrids based on the ability of SWCNTs to quench conventional organic fluorophores.⁴⁹ We first constructed (GT)₆- or (GT)₃₀-SWCNTs with a Cy3 dye attached to the 5' end of the DNA strand. The initially quenched fluorophore could be restored to a brightly fluorescent state via displacement from the SWCNT surface by a competing molecule (Figure 3. 3a). Note, even partial displacement of the DNA strand can accomplish this process, thus Cy3 dequenching kinetics of the two prepared hybrids are similar despite unequal displacement kinetics by sodium deoxycholate (SDC) determined from NIR fluorescence (Figure 3. 3b).³³ When introduced to macrophage cells in the same 30-minute pulse method, we observed substantially different dequenching behavior between the two sequences (Figure 3. 3c). The Cy3-(GT)₆-SWCNTs significantly dequenched inside of the cells, reaching a maximum intensity 4 hours after the pulse (Figure 3. 3d) and decreased to its initial intensity after 24 hours. In contrast, dequenching was not observed in the Cy3-(GT)₃₀-SWCNTs at any point, resulting in statistically significant differences in the dequenching behavior of the DNA-SWCNTs, *i.e.* either partial or full displacement of the DNA from the SWCNTs, within the first six

hours.

Figure 3. 3. Intracellular stability of DNA-SWCNT hybrids. **(a)** Schematic of experimental design. Cy3-DNA is quenched when wrapping is intact on SWCNTs, but highly fluorescent once displaced. **(b)** Normalized intensity increase as a function of time after Cy3-DNA is displaced with SDC. **(c)** Overlaid Cy3-DNA and white light images of RAW 264.7 pulsed with Cy3-(GT)₆ or Cy3-(GT)₃₀-SWCNTs for 30 minutes. Scale bar = 10 μ m. **(d)** Average fluorescence intensities ($n \geq 14$ cells) normalized to 0-hour intensity. Error bars represent mean $\pm$ s.d. Five-pointed stars represent significance between Cy3-(GT)₆ and Cy3-(GT)₃₀, and six pointed stars represent significance versus initial intensities. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ according to two-tailed two-sample t-test).

While the observed NIR fluorescence of SWCNTs can be modulated by both concentration and local-environment,^{16, 48, 50} certain Raman signatures of pristine SWCNTs depend only on concentration,⁵¹⁻⁵⁴ allowing for all SWCNT chiralities in a sample to be represented regardless of fluorescence ability. Therefore, we assessed the localized intracellular concentrations of SWCNTs using confocal Raman microscopy. Small regions

were scanned in 0.5 μm intervals to obtain Raman maps of macrophages pulsed with 1 mg/L (GT)₆- or (GT)₃₀-SWCNTs for 30 minutes (Figure 3. 4a). The intensity of the G-band spectral feature, indicative of sp^2 carbon,^{52, 53} was correlated to known SWCNT concentrations in the construction of a calibration curve in order to obtain a mass of SWCNTs per analyzed cell (Figure S3. 6).

Figure 3. 4. SWCNT concentration maps determined by confocal Raman microscopy. **(a)** Representative confocal Raman microscopy images showing G-band intensity and white light images of RAW 264.7 cells pulsed with (GT)₆- or (GT)₃₀-SWCNTs for 30 minutes. Color map represents local SWCNT pixel concentration derived from G-band intensity calibration (60x objective). Scale bar = 10 μm . **(b)** Average SWCNT concentration ($n \geq 4$ cells) calculated from total pixel concentration within cellular ROIs. **(c)** Percent of SWCNT dose internalized compared to the initial incubated concentration, determined from cell media supernatant concentration after a 30-minute DNA-SWCNT pulse ($n = 10$). **(d)** Concentration of exocytosed SWCNTs in cell supernatant 24 hours after pulse ($n = 4$). **(e)** Representative confocal Raman G-band intensity maps overlaid on white light images of macrophages 24 hours after a 30-minute pulse of (GT)₆- or (GT)₃₀-SWCNTs. Indicated cells were treated with exocytosis inhibitors Bafilomycin A1 (200 nM), Nocodazole (2 μM), Brefeldin A (500 nM), or Exo1 (50 μM) 2 hours following DNA-SWCNT pulse. Color map represents local SWCNT pixel concentration derived from G-band intensity calibration (10x objective). Scale bar = 75 μm . **(f)** Average concentration of SWCNT-containing pixels from all confocal Raman area scans described in **(e)**, ($n \geq 3$ area scans).

Error bars are represented as mean $\pm$ s.d. for all. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, according to two-tailed two-sample t-test).

Although the local concentrations varied greatly within a single cell, on average the cells pulsed with (GT)₆-SWCNTs had more than twice the initial intracellular SWCNT weight than those incubated with (GT)₃₀-SWCNTs (Figure 3. 4b). After 24 hours of additional incubation in SWCNT-free cell media, the internal SWCNT concentration of cells dosed with (GT)₆-SWCNTs decreased by more than 75%, while those dosed with (GT)₃₀-SWCNTs displayed statistically similar initial and final concentrations. While cellular uptake of nanoparticles can be influenced by a multitude of factors, we attribute the higher uptake of (GT)₆-SWCNTs to their higher overall density of DNA per SWCNT as compared to (GT)₃₀-SWCNTs,³¹ increasing the probability of interactions between DNA and cellular membrane proteins and thus leading to more nanotubes per engulfing phagosome. Conversely, we surmise that changes in the physical identity of internalized (GT)₆-SWCNTs are inducing the macrophages to exocytose this sample more rapidly than the stable (GT)₃₀-SWCNTs.

To corroborate the unexpected results from confocal Raman microscopy, we performed solution-based Raman spectroscopy to determine the SWCNT concentrations in the supernatants at time 0 and 24 hours, representing the delivered dose and the amount of exocytosed SWCNTs, respectively. The relative delivered dose, calculated as the percent decrease in supernatant G-band intensity after a 30-minute pulse incubation with the cells, confirmed that cells internalized a significantly higher amount of (GT)₆-SWCNTs than (GT)₃₀-SWCNTs (Figure 3. 4c). Furthermore, additional agreement with Raman microscopy data was observed at 24 hours when significantly more (GT)₆-SWCNTs were

found in the supernatant as compared to (GT)₃₀-SWCNTs (Figure 3. 4d). We believe these results verified that the decreased intracellular concentration of (GT)₆-SWCNTs after 24 hours was the result of exocytosis and not cell-mediated degradation of the SWCNT material.

Finally, we sought to better understand the mechanisms dictating retention versus exocytosis by identifying the main pathway in which (GT)₆-SWCNTs were being expelled from the cells. Typically, the fate of phagocytosed nanomaterials contained within lysosomes is either regulated secretion, in which the contents are further processed and excreted from the Golgi apparatus, or lysosomal exocytosis via direct fusion with the cell membrane.⁵⁵⁻⁵⁷ Therefore, we devised an assay to compare the intracellular DNA-SWCNT concentration of macrophages after treatment with specific pathway inhibiting compounds. Bafilomycin A1 and Nocodazole, both of which inhibit lysosomal exocytosis,^{58, 59} induced retention of (GT)₆-SWCNTs within cells at significantly higher average concentrations than the control after 24 hours (Figure 3. 4e-f), while little effect was seen on the average concentrations of (GT)₃₀-SWCNTs. Conversely, Brefeldin A and Exo1, inhibitors of Golgi-mediated exocytosis,^{60, 61} did not cause a significant increase in average concentration for either DNA-SWCNT hybrid, suggesting that the main clearance mechanism for DNA-SWCNTs is through lysosomal exocytosis.

One of the main functions of lysosomal exocytosis is to secrete various biomolecules such as proteins, enzymes, or antigens for intercellular communication and illicit an immune response from nearby cells if necessary.^{42, 62} Studies have shown that while SWCNTs with various types of surface functionalization can reduce or prevent cytotoxicity,⁶³ pristine SWCNTs are recognized as pathogenic substances upon interaction

with Toll-like receptors present on the cell membrane of macrophages,⁶⁴ leading to the secretion of inflammatory cytokines as a mechanism of defense.⁶⁵ Therefore, we believe that the ability of the lysosomal environment to remove the DNA from (GT)₆-SWCNTs causes the cell to identify the altered nanomaterial as it would a non-functionalized SWCNT (Figure 3. 5a). Once the cell has recognized this material as a foreign body, excretion from the cell via lysosomal exocytosis is initiated in order to illicit an immune response from nearby cells, resulting in a diminished intracellular SWCNT concentration. Conversely, the increased stability provided by a longer DNA wrapping prevents major alterations from occurring in the lysosomal environment and avoids triggering exocytosis, resulting in a high degree of cellular retention (Figure 3. 5b).

Figure 3. 5. Schematic depicting the DNA length-dependent intracellular processing of DNA-SWCNTs. **(a)** (GT)₆-SWCNTs are internalized into macrophages in large amounts and localize to the lysosomes. There, biomolecules displace DNA from the SWCNT surface and induce lysosomal exocytosis from the cells. **(b)** (GT)₃₀-SWCNTs are also

located to the lysosomes after internalization into the cells but do not experience DNA displacement. These DNA-SWCNTs with enhanced integrity are retained within the cells.

3. 4. Conclusions

We propose that the intracellular processing and ultimate fate of (GT)_n-SWCNTs are controlled by the differential stabilities of the hybrid nanomaterials in the lysosomal environment, which correlate strongly to the length of a given DNA strand. The observed intracellular fluorescence intensities were shown to increase with increasing oligonucleotide length, while only the shortest DNA-SWCNTs (*i.e.* (GT)₆) displayed instabilities in NIR fluorescence spectra in time. We have shown that (GT)₃₀-SWCNTs are mostly retained within the cells over 24 hours with minimal exocytosis, while (GT)₆-SWCNTs expelled more than 75% of the internalized cargo over the same time period despite nearly a two-fold higher amount of initial uptake. The correlation between an increase in emission energy and the dequenching of Cy3-(GT)₆ strongly suggests that competitive molecular adsorption to the SWCNT sidewall results in a destabilized structure within the lysosome, increasing the probability of complete DNA displacement or degradation from the SWCNT. Without the biocompatibility afforded by the DNA wrapping, the cell is able to recognize a SWCNT as a foreign pathogenic substance and subsequently secrete its lysosomal contents. These findings accentuate the necessity of biocompatible stability when designing any carbon nanotube-based biosensors while highlighting their sensitivity to small changes in surface chemistry.

3. 5. Materials and Methods

DNA-SWCNT Sample Preparation: Raw single-walled carbon nanotubes

produced by the HiPco process (Nanointegris) were used throughout this study. For each dispersion, 1 mg of raw nanotubes was added to 2 mg of (GT)_n (where $n = 6, 9, 12, 15$, or 30) oligonucleotide (Integrated DNA Technologies), suspended in 1 mL of 0.1M NaCl (Sigma-Aldrich), and ultrasonicated using a 1/8" tapered microtip for 30 min at 40% amplitude (Sonics Vibracell VCX-130; Sonics and Materials). The resultant suspensions were ultra-centrifuged (Sorvall Discovery M120 SE) for 30 min at 250,000 $\times g$ and the supernatant was collected. Concentrations were determined using a UV/vis/NIR spectrophotometer (Jasco, Tokyo, Japan) and the extinction coefficient of $A_{910} = 0.02554 \text{ L mg}^{-1} \text{ cm}^{-1}$.¹⁵

Cell Culture: RAW 264.7 TIB-71 cells (ATCC, Manassas, VA, USA) were cultured under standard incubation conditions at 37 °C and 5% CO₂ in cell culture medium containing sterile filtered high-glucose DMEM with 10% heat-inactivated FBS, 2.5% HEPES, 1% L-glutamine, 1% penicillin/ streptomycin, and 0.2% amphotericin B (all acquired from Gibco). For all cell-related studies, cells were allowed to grow until 90% confluency and used up to the 20th passage.

Near-Infrared Fluorescence Microscopy of Live Cells: A near-infrared hyperspectral fluorescence microscope, similar to a previously described system,¹⁵ was used to obtain fluorescence images and hyperspectral data within live cells. In short, a continuous 730 nm diode laser with 1.5 W output power was injected into a multimode fiber to produce an excitation source, which was reflected on the sample stage of an Olympus IX-73 inverted microscope equipped with a 20X LCPlan N, 20x/0.45 IR objective (Olympus, USA) and a stage incubator (Okolab) to maintain 37 °C and 5% CO₂ during imaging. Emission was passed through a volume Bragg Grating and collected with

a 2D InGaAs array detector (Photon Etc.) to generate spectral image stacks. For live cell experiments, cells were seeded into tissue culture treated 96-well plates (Fisher Scientific) at a final concentration of 50,000 cells/ well and allowed to culture overnight in an incubator. The media was removed from each well, replaced with 1 mg/L each (GT)_n-SWCNT diluted in media, and incubated for 30 minutes (pulsed) to allow for internalization into the cells. After this pulse, the SWCNT-containing media was removed, the cells were rinsed 3X with sterile PBS (Gibco) and fresh media was replenished. Well plates were mounted on the hyperspectral microscope to obtain broadband images, transmitted light images, and fluorescence hyperspectral images at each given time point. Hyperspectral data were processed and extracted using custom codes written with Matlab software. All Gaussian curve fits were generated using OriginPro 2018.

Solution-Based Fluorescence Dequenching Assay: Cy3-(GT)₆- or Cy3-(GT)₃₀ oligonucleotides were purchased from Integrated DNA Technologies and used in the creation of DNA-SWCNTs (see above). After ultrasonication and ultracentrifugation, the Cy3-DNA-SWCNTs were filtered 3 times using 100 kDa Amicon centrifuge filters (Millipore) to remove free Cy3-DNA from solution, diluted to 2.5 mg/L, and 1 mL was placed in a plastic cuvette under magnetic stirring. The fluorescence intensity of each sample was obtained in 1-second intervals for 3 minutes using a Perkin Elmer LS 55 fluorescence spectrometer set to 532 nm excitation and 569 nm emission with 3 nm bandwidth. A 10 µL aliquot of a 10% sodium deoxycholate solution (Sigma-Aldrich) was spiked into the Cy3-DNA-SWCNTs after a baseline intensity was established for a final concentration of 0.1% SDC in order to temporally displace the Cy3-DNA from the SWCNTs as previously described.³³

Visible Fluorescence Microscopy in Live Cells: Cy3-(GT)₆-SWCNTs and Cy3-(GT)₃₀-SWCNTs were first filtered 3 times using 100kDa Amicon centrifuge filters (Millipore) to remove free Cy3-DNA from solution. The cells were seeded onto 35 mm glass-bottom petri dishes (MatTek) to a final concentration of 500,000 cells/dish and allowed to culture overnight in an incubator. The media was removed from each well, replaced with 1 mg/L of filtered Cy3-(GT)₆-SWCNTs or Cy3-(GT)₃₀-SWCNTs diluted in media, and incubated for 30 minutes to allow internalization into the cells. The SWCNT-containing media was removed, the cells were rinsed 3X with sterile PBS (Gibco), and fresh media was replenished for each sample. The petri dishes were mounted in a stage incubator (Okolab) on an Olympus IX-73 inverted microscope with a UApo N 100x/1.49 oil immersion objective for epifluorescence imaging with a U-HGLGPS excitation source (Olympus) filtered through a Cy3 filter cube. The fluorescence images were analyzed by extracting average fluorescence intensity values of individual cell ROIs using ImageJ.

Confocal Raman Microscopy: Cells were seeded into 35mm glass bottom microwell dishes (MatTek) to a final concentration of 500,000 cells/ dish and allowed to culture overnight in incubator. The media was removed from each well, replaced with 1 mg/L (GT)₆-SWCNT or (GT)₃₀-SWCNT diluted in media, and pulsed for 30 minutes to allow internalization into the cells. The SWCNT-containing media was removed, the cells were rinsed 3X with sterile PBS (Gibco), and fresh media was replenished. The 0-hour samples were immediately fixed using 4% paraformaldehyde in PBS for 10 minutes, rinsed 3X with PBS, and covered with PBS to retain an aqueous environment during imaging. The 24-hour samples were later fixed using the same procedure. The cells were imaged using an inverted WiTec Alpha300 R confocal Raman microscope (WiTec, Germany)

equipped with a Nikon CFI-Achro 60x/0.8 air objective, a 785nm laser source set to 35mW sample power, and collected with a CCD detector through a 600 lines/mm grating. The Raman spectra were obtained in 0.5x0.5 μm intervals with 1s integration time to construct hyperspectral Raman area scans of cellular regions. A calibration curve was obtained by recording spectra of known SWCNT concentrations serially diluted in a single pixel volume with identical acquisition settings. Each spectrum was averaged over 20 scans. A global background subtraction and cosmic-ray removal was performed using Witec Control 5.0 software on all acquired confocal Raman data and G-band maximum intensities were extracted and correlated with known concentrations to produce a linear curve fit using OriginPro 2018 analysis software. The cellular SWCNT concentration data were produced by relating the G-band linear equations to each SWCNT-containing pixel and intracellular concentrations were obtained in individual cell ROIs with the correlation $\frac{\text{SWCNT weight}_{\text{cell}}}{\text{total ROI SWCNT concentration} * \text{total SWCNT pixel volume}} =$

Solution-based Raman Spectroscopy: RAW 264.7 cells were seeded into tissue culture treated 96-well plates (Fisher Scientific) at a final concentration of 50,000 cells/well and allowed to culture overnight in an incubator. The media was removed from each well, replaced with 200 μL of 1 mg/L each (GT)_n-SWCNT diluted in media, and incubated for 30 minutes (pulsed) to allow for internalization into the cells. After this pulse, the SWCNT-containing media was collected, the cells were rinsed 3X with sterile PBS (Gibco) and 200 μL of fresh media was replenished. 24-hours later, the supernatant was again collected from the cells. All supernatant was placed into new 96-well plates and Raman spectra were obtained using a WiTec Alpha300 R confocal Raman microscope (WiTec,

Germany) equipped with a Zeiss Epiplan-Neofluar 10x/0.25 objective, a 785nm laser source set to 35mW sample power, and collected with a CCD detector through a 600 lines/mm grating. A calibration curve was obtained by recording spectra of known SWCNT concentrations serially diluted in a single pixel volume with identical acquisition settings. A global background subtraction and cosmic-ray removal was performed using Witec Control 5.0 software on all acquired confocal Raman data and G-band maximum intensities were extracted and correlated with known concentrations to produce a linear curve fit using OriginPro 2018 analysis software. The intensity of the G-band was extracted from each spectrum and related to G-band linear fit equations to determined average supernatant SWCNT concentrations.

Pharmacological Inhibition of Exocytosis Pathways: RAW 264.7 cells were cultured and dosed with (GT)₆- or (GT)₃₀-SWCNTs following the same procedure previously described, however 2 hours after SWCNT removal cells were spiked with either 200 nM Bafilomycin A1,⁵⁸ 2 μ M Nocodazole,⁵⁹ 500 nM Brefeldin A,⁶¹ 50 μ M Exo1,⁶⁰ or an equal volume of media. At 24 hours after the initial SWCNT dose (22 hours post inhibitor treatment), cells were fixed using 4% paraformaldehyde in PBS for 10 minutes, rinsed 3X with PBS, and covered with PBS to retain an aqueous environment during imaging. Large cellular regions were scanned in 10 μ m intervals using a WiTec Alpha300 R confocal Raman microscope (WiTec, Germany) equipped with a Zeiss Epiplan-Neofluar 10x/0.25 objective, a 785nm laser source set to 35mW sample power, and collected with a CCD detector through a 600 lines/mm grating with a 0.5s integration time. A calibration curve was obtained by recording spectra of known SWCNT concentrations serially diluted in a single pixel volume with identical acquisition settings. A global background

subtraction and cosmic-ray removal was performed using Witec Control 5.0 software on all acquired confocal Raman data and G-band maximum intensities were extracted and correlated with known concentrations to produce a linear curve fit using OriginPro 2018 analysis software. The cellular SWCNT concentration data were produced by relating the G-band linear equations to each SWCNT-containing pixel and average cellular SWCNT concentrations were extracted from each area scan using a custom Matlab script.

Statistical Analysis: All statistical measures for hypothesis testing were carried out using two-sample two-tailed unequal variance t-tests in Microsoft Office Excel 2016. All curve fitting and related statistics were performed in OriginPro 2018.

3. 6. Acknowledgements

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3. 7. References

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Chapter 4

DNA Sequence Mediate Apparent Length Distribution in Single-Walled Carbon Nanotube

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4. 1. Summary

Single-walled carbon nanotubes (SWCNTs) functionalized with short single-stranded DNA have been extensively studied within the last decade for biomedical applications due to the high dispersion efficiency and intrinsic biocompatibility of DNA as well as the photostable and tunable fluorescence of SWCNTs. Characterization of their physical properties, particularly their length distribution, is of great importance regarding their application as a bioengineered research tool and clinical diagnostic agent. Conventionally, atomic force microscopy (AFM) has been used to quantify the length of DNA-SWCNTs by depositing the hybrids onto an electrostatically charged flat surface. Here, we demonstrate that hybrids of DNA-SWCNTs with different oligomeric DNA sequences ((GT)₆ and (GT)₃₀) differentially deposit on the AFM substrate, resulting in significant inaccuracies in the reported length distributions of the parent solutions. Using a solution-based surfactant exchange technique, we placed both samples into a common surfactant wrapping and found identical SWCNT length distributions upon surface deposition. Additionally, by spin-coating the surfactant wrapped SWCNTs onto a substrate, thus mitigating effects of electrostatic interactions, we found length distributions that did not depend on DNA sequence but were significantly longer than electrostatic deposition methods, illuminating the inherent bias of the surface deposition method. Quantifying the coverage of DNA molecules on each SWCNT through both absorbance spectroscopy and direct observation, we found that the density of DNA per SWCNT was significantly higher in short (GT)₆-SWCNTs (length < 100 nm) compared to long (GT)₆-SWCNTs (length > 100 nm). In contrast, we found no dependence of the DNA density on SWCNT length in (GT)₃₀-SWCNT hybrids. Thus, we

attribute differences in the observed length distributions of DNA-SWCNTs to variations in electrostatic repulsion induced by sequence-dependent DNA density.

4. 2. Introduction

Single-walled carbon nanotubes (SWCNTs) with engineered wrappings¹⁻⁴ have recently been developed and utilized in various disparate fields ranging from additives that strengthen material composites⁵⁻⁸ to biomedical applications including targeted anti-cancer drug delivery⁹⁻¹¹, near-infrared (NIR) optical biosensing,¹²⁻¹⁴ and biological imaging.¹⁵⁻¹⁸ Of significant interest, SWCNTs exhibit intrinsic photoluminescence (fluorescence) that is photostable, tunable, and sensitive to its local environment.^{19, 20} Unfortunately, covalent functionalization is known to negatively affect SWCNT electrical and optical properties,²¹ thus, alternative non-covalent methods are preferred. Certain amphiphilic biomolecules such as DNA,^{4, 14, 22} peptides,^{23, 24} proteins,^{25, 26} or lipids,^{27, 28} have been shown to effectively solubilize SWCNTs, in addition to imparting enhanced biocompatibility and preserving their desirable fluorescence by non-covalently adsorbing onto the aromatic side-wall of the SWCNT.²⁹

Having both hydrophobic bases and a hydrophilic backbone, single-stranded DNA has been demonstrated to non-covalently attach to the outside of the SWCNTs by hydrophobic and π - π stacking interactions and act as a highly-effective dispersant for SWCNTs.⁴ This dispersion process generally involves probe-tip sonication to impart enough energy in order to break up initially bundled SWCNTs²⁹ with the off-target effect of cutting the SWCNTs to random lengths.³⁰ Both empirical studies^{31, 32} and molecular dynamics simulations³³ have shown that DNA helically wraps the SWCNTs in aqueous solution, and

further that this structure is preserved when the DNA-SWCNT hybrid is dried onto a surface.³⁴ Interestingly, while longer oligonucleotides have a stronger binding affinity to SWCNTs, as probed through absorption spectroscopy, atomic force microscopy, nanoparticle fluorescence spectroscopy and molecular dynamics simulations, certain short sequences of DNA recognize specific chiralities of SWCNTs and permit their separation from an ensemble solution.³⁵

There have been many recent studies demonstrating the utilization of DNA-SWCNT hybrids in biomedical applications.^{12-14, 28, 36-38} Critical to their adoption as a standard biological research tool is a thorough characterization of their physical properties, namely their length distribution. The length of a SWCNT directly correlates to its photoluminescence (fluorescence) intensity.³⁹ For biological imaging and sensing applications, quantitative measurements rely on accurate determinations of the SWCNT length. For example, DNA-wrapped SWCNTs have recently been introduced as intracellular sensors of lipid accumulation both in live cells¹³ and in mice.⁴⁰ In the development of these and other types of SWCNT-based sensors, accurate length determinations of SWCNTs provide the most precise quantification of targeted analytes. Further, the toxicological response of SWCNTs can be more accurately predicted given knowledge of their average length and length distribution.^{41, 42}

The conventional method for determining the length distribution of a population of DNA-SWCNTs is to deposit the hybrids onto an atomically flat surface, e.g. freshly cleaved mica, and image them with atomic force microscopy (AFM) techniques.⁴ However, it is unknown whether this adsorbed fraction of DNA-SWCNTs is representative of the entire solution sample. A search of the literature has uncovered ~20 publications within

the last decade that have used this particular approach for length determination.^{4, 34, 41, 43-57} Herein, we demonstrate that the apparent length distribution of DNA-SWCNTs, as determined by conventional AFM imaging techniques, is highly dependent upon the sequence of DNA that is used to wrap the SWCNTs. In particular, under identical sample preparation conditions, oligomeric DNA with two different sequence lengths, (GT)₆ and (GT)₃₀, gave apparent length distributions of 150 ± 8 and 67 ± 7 nm, respectively. By transferring the hybrids into a common wrapping through the use of sodium deoxycholate (SDC)-induced DNA displacement, we show that the length distributions of the two samples converge to a statistically identical value. Spin-coating of these samples onto a substrate, thereby reducing the electrostatic interactions between the SWCNTs and the substrate, gave length distributions that did not depend on DNA sequence, but were longer than electrostatically deposited samples. Furthermore, by quantifying the number of DNA sequences on the individual SWCNTs, we found that shorter (GT)₆-SWCNTs (length < 100 nm) displayed significantly higher densities of DNA than any other sub-population of examined DNA-SWCNTs. Finally, we quantified the exact weights of DNA and SWCNT in each sample with absorbance spectroscopy and propose a model to explain the differential rates of binding to the AFM imaging surface that depend on SWCNT length and DNA loading.

4. 3. Results and Discussion

Singly-dispersed hybrids of SWCNTs and single-stranded (GT)₆, *i.e.* GTGTGTGTGTGT, or ss(GT)₃₀ were prepared by previously reported sonication and ultracentrifugation techniques.²⁰ In order to determine the length distribution of each sample, a solution of DNA-SWCNTs was exposed to an ultra-flat surface (freshly-cleaved mica)

for 4 minutes (herein referred to as “deposited”), the surface was washed, and then imaged with an atomic force microscope. Figures 4. 1a-b show representative AFM images obtained from (GT)₆-SWCNTs or (GT)₃₀-SWCNTs, respectively.

Figure 4. 1. DNA-SWCNTs deposited onto mica. AFM images of (a) (GT)₆-SWCNTs and (b) (GT)₃₀-SWCNTs. (c) Histograms for length distributions of (GT)₆-SWCNTs and (GT)₃₀-SWCNTs. (d) The corresponding box and whisker plots to compare the average SWCNT lengths from each of four AFM images per sample for (GT)₆-SWCNTs and (GT)₃₀-SWCNTs. The boxes represent the minimum, maximum, and mean values from n=4 images, each image containing ~400 DNA-SWCNTs. The whiskers represent 25-75 percentile data. A two-sample t-test was performed on the image-averaged data (***, p<0.001).

From initial inspection of the images, it is clear that the length distribution of deposited SWCNTs was larger in the (GT)₆ sample than that of (GT)₃₀. To quantify the

difference, manual counting of the SWCNT lengths was performed on ~1600 SWCNTs from each sample. The derived length distributions are presented in Figures 4. 1c and S4. 1a and confirm that the distribution for (GT)₆-SWCNTs was shifted to longer SWCNT lengths compared to that of the (GT)₃₀-SWCNT distribution. Furthermore, the average SWCNT lengths from each of four AFM images per sample, each image containing ~400 SWCNTs, were compared. These data are presented in Figure 4. 1d and show that the average length of deposited (GT)₆-SWCNTs and (GT)₃₀-SWCNTs were 150 ± 8 and 67 ± 7 , respectively (Figure S4. 2a shows the data from four images combined together). This difference demonstrates that deposited (GT)₆-SWCNTs were significantly longer (two-sample t-test, $p < 0.001$) than deposited (GT)₃₀-SWCNTs.

The previous result regarding apparent length distributions of DNA-SWCNTs that depended upon DNA sequence gave rise to one of two hypotheses: 1) the two samples were intrinsically different and that the length distributions of deposited DNA-SWCNTs were representative of the parent solutions or 2) the differing DNA sequences introduced an artifact in the binding process leading to length distributions that differed from the parent solutions. To identify the cause of the sequence-dependent difference in apparent lengths, we used a solution-based surfactant exchange technique that displaced the DNA from the two samples and placed them both into a common surfactant wrapping.²⁰ Similar to the DNA-SWCNT samples, the two DNA-displaced SDC-SWCNT samples, denoted as (GT)₆ to SDC and (GT)₃₀ to SDC, were deposited onto mica and imaged with AFM (Figure 4. 2a-b).

Figure 4. 2. DNA-displaced SDC-SWCNTs deposited onto mica. AFM images of (a) (GT)₆-displaced SDC-SWCNTs and (b) (GT)₃₀-displaced SDC-SWCNTs. (c) Histograms for length distributions of (GT)₆-displaced SDC-SWCNTs ((GT)₆ to SDC) and (GT)₃₀-displaced SDC-SWCNTs ((GT)₃₀ to SDC). (d) The corresponding box and whisker plots to compare the average SWCNT lengths from each of four AFM images per sample for

(GT)₆-displaced SDC-SWCNTs and (GT)₃₀-displaced SDC-SWCNTs. The boxes represent the minimum, maximum, and mean values from n=4 images, each image containing ~150 SDC-SWCNTs. The whiskers represent 25-75 percentile data. Box and whisker plots to compare the average SWCNT lengths from each of four AFM images per sample for (e) (GT)₆ -SWCNTs and (GT)₆-displaced SDC-SWCNTs, and (f) (GT)₃₀ -SWCNTs and (GT)₃₀-displaced SDC-SWCNTs. The boxes represent the minimum, maximum, and mean values from n=4 images. The whiskers represent 25-75 percentile data. A two-sample t-test was performed on the image-averaged data (*, p<0.05, **, p<0.01).

The displacement of DNA by SDC is confirmed both by blue-shifts in the near-infrared absorbance peaks of the SWCNTs³⁵ (Figure S4. 3) and by the irregular pattern of SDC molecules on the SWCNTs in contrast to the regular pattern (helical wrapping) of DNA on the SWCNTs.³⁴ Despite an increased background height, presumably due to excess surfactant bound to the mica, it is visually evident that the two samples had similar distributions of SWCNT lengths. The length measurements for DNA-displaced SDC-SWCNTs, presented as overall length distributions (Figure 4. 2c and S4. 1b) and image-averaged data (Figure 4. 2d), each image containing ~150 SWCNTs, confirm the lack of significant difference (two-sample t-test) between the two surface-bound SDC-SWCNT samples (Figure S4. 2b presents the data from four images combined together). The data affirm the hypothesis that the apparent length distributions of deposited SWCNTs are influenced by both DNA sequence and wrapping type, *i.e.* DNA vs. surfactant (Figure 4. 2e-f).

It has been reported by Weisman *et al.* that AFM imaging of spin-coated SWCNTs dispersed in surfactant accurately represents the solution phase in terms of SWCNT lengths, since all SWCNTs from the parent sample are placed onto the substrate.⁵⁸ Therefore, we repeated length characterizations of DNA-displaced SDC-SWCNT samples that were spin-coated onto pre-cleaned silicon wafers (Figure S4. 4). Despite this alternative

method of placing SWCNTs on a surface, we observed no significant dependence on DNA sequence (Figure S4. 4a-d). Notably, we observe significant increases in mean SWCNT lengths compared to deposited DNA-displaced SDC-SWCNTs (Figure S4. 4e-f). We hypothesize that the increases stem from mitigating the electrostatic interactions between the SWCNTs and the substrates, thereby yielding more accurate length distributions.

To explain the difference in length distributions of deposited DNA-SWCNTs, we hypothesize that differential interactions exist between SWCNTs and DNA of varying sequence and further that these interactions mediate the process of surface adsorption. We examined the height profiles along single SWCNTs in higher resolution AFM images in order to quantify the coverage from DNA molecules interacting with individual SWCNTs (Figure 4. 3a-b for representative (GT)₆-SWCNT and Figure 4. 3c-d for representative (GT)₃₀-SWCNT). As demonstrated previously,³⁴ the absolute number of DNA molecules and the density of DNA coverage on each SWCNT was quantified by counting the peaks in the height profiles. It is worth noting that each peak in DNA-SWCNT height profile could arise from either each turn of helically wrapped DNA around the SWCNT or each DNA molecule on the SWCNT, depending on the length of the DNA strand.³⁴ The number of turns made by a single DNA around a single SWCNT can be calculated by assuming that rolling a rectangle, so that its diagonal (of length l) makes exactly one helical turn, forms a cylinder which can be wrapped with one helical turn of DNA. The length of the diagonal (l) can be calculated from Pythagorean Theorem³⁴: $l = \sqrt{h^2 + (\pi d)^2}$, where h is the average peak to peak distance quantified from high resolution AFM images (Figure S4. 5a-b) and d is the average diameter of HiPco SWCNTs (1.1 nm).⁵⁹ The total length of the stretched DNA molecule (L) is number of its turns around the SWCNT (n) times l ($L =$

nl). “L” is calculated by assuming a distance of 0.7 nm between phosphorus atoms on the DNA backbone (L is 8.4 nm for a 12 mer).³⁴ Thus, the number of the turns made by a single DNA around a single SWCNT was calculated to be 0.6 and 2.8 for (GT)₆-SWCNTs and (GT)₃₀-SWCNTs, respectively. These numbers imply that the (GT)₆ strand is not long enough to have a complete turn and shows up as a single peak in the height profiles whereas (GT)₃₀ makes two complete turns and one partial turn around the SWCNTs, and appears as three peaks per each molecule in the height profiles.

Figure 4. 3. Height profiles along the representative individual SWCNTs. (a) and (b) (GT)₆-SWCNT. (c) and (d) (GT)₃₀-SWCNT. Each bright spot on the SWCNT represents a peak in the height profile.

We plotted the number of the peaks versus length of each examined SWCNT in the two samples (Figure 4. 4a). Positive linear correlations between the number of the peaks

and SWCNT length were observed (Pearson correlation coefficients of 0.832 and 0.889 for (GT)₆-SWCNTs and (GT)₃₀-SWCNTs, respectively), suggesting a consistent density of DNA along the SWCNT. A single ratiometric parameter could be derived for each DNA-SWCNT by dividing the number of the peaks by the length of the SWCNT, *i.e.* [count / nm], as a measure for the DNA density on the SWCNT side-wall. Denoted herein as “N/L”, we present total N/L distributions in Figure 4. 4b, and image-averaged N/L data (each image containing 16 data) in Figure 4. 4c (Figure S4. 2d demonstrates the data from four images combined together). On average, the surface coverage of DNA on SWCNTs is significantly higher ($p < 0.01$) in (GT)₆-SWCNTs than that of (GT)₃₀-SWCNTs.

Figure 4. 4. (a) Number of peaks on single SWCNT versus length of each SWCNT in (GT)₆-SWCNTs and (GT)₃₀-SWCNTs, (b) Overlaid histograms for the distribution of N/L for (GT)₆-SWCNTs and (GT)₃₀-SWCNTs, (c) Box and whisker plots to compare the

averages of the N/L ratios obtained from five images for each sample. The boxes represent the minimum, maximum, and mean values from $n=5$ images, each image containing ~ 16 SWCNTs. The whiskers represent 25-75 percentile data. Two-sample t-tests were performed on the image-averaged data (**, $p<0.01$).

To confirm this finding of differential DNA coverage from the AFM images, we quantified the actual weights of DNA and SWCNTs in aqueous samples using absorbance spectroscopy and the previously utilized surfactant exchange method. Using extinction coefficients for DNA and SWCNTs at 260 nm⁶⁰ and 910 nm,⁶¹ respectively, the absolute weights were determined and the weight ratios of DNA to SWCNT are reported in Figure 4. 5. This ratio in (GT)₆-SWCNT sample is significantly higher than that of (GT)₃₀-SWCNT, confirming the finding from comparing the N/L of the two samples.

Figure 4. 5. The ratio of the total weight of wrapped DNA to weight of SWCNTs in solutions of (GT)₆-SWCNT and (GT)₃₀-SWCNT. Mean values were obtained from $n=3$ measurements per sample. Two-sample t-tests were performed on the data (**, $p<0.01$).

Upon closer inspection of the scatterplots found in Figure 4. 4a, we detect distinct subpopulations in the data. By dividing the data into two populations distinguished by the length of the SWCNTs, *e.g.* SWCNTs greater than or less than 100 nm, respectively, we

uncovered significant differences in the density of DNA per SWCNT (Figure 4. 6). While we found no dependence of the N/L ratios on SWCNT length in (GT)₃₀-SWCNT hybrids, we observed that (GT)₆-SWCNTs with lengths less than 100 nm have an average of 27 % higher DNA density ($p < 0.01$).

Figure 4. 6. N/L ratios for two subgroups distinguished by the length of the SWCNTs (Length < 100 nm and Length > 100 nm) for (GT)₆-SWCNTs and (GT)₃₀-SWCNTs.

We believe that this result endorses the DNA sequence-dependent discrepancies of length distributions in deposited DNA-SWCNTs due to the following mechanism: with permanent negative charges at neutral pH, non-covalent complexation of DNA with SWCNTs results in a negatively charged hybrid with charge density that scales with DNA density (N/L ratio). As a result, the electrostatic repulsion between the negatively charged AFM substrate (mica) and shorter (GT)₆-SWCNTs (length < 100 nm) in water is significantly larger than that of the substrate and longer (GT)₆-SWCNTs (length > 100 nm). The longer (GT)₆-SWCNTs subsequently bind to the AFM substrate at a faster rate compared to shorter (GT)₆-SWCNTs within the limited time interval (5 minutes) allowed for binding.

In contrast, since the DNA densities in (GT)₃₀-SWCNTs do not depend on the SWCNT length, the electrostatic repulsion between short and long (GT)₃₀-SWCNTs and the AFM substrate will be similar. Here, we propose that the shorter (GT)₃₀-SWCNTs will bind to the AFM substrate with a faster rate due to their intrinsically higher diffusion coefficients.⁵⁸ A schematic illustrating this differential binding process is presented in Figure 4. 7. We emphasize the difference between DNA density on SWCNT, and the number of the turns of each DNA molecule around SWCNT.

Figure 4. 7. Illustration of the differential binding process of DNA functionalized SWCNTs on mica substrates. (a) Short (GT)₆-SWCNTs have higher DNA density compared to long (GT)₆-SWCNTs. This causes stronger electrostatic repulsion between short (GT)₆-SWCNTs and the negatively charged substrate, leading to higher fraction of long (GT)₆-SWCNTs binding. (b) DNA density on short and long (GT)₃₀-SWCNTs is the same. Increased binding of short (GT)₃₀-SWCNTs compared to long (GT)₃₀-SWCNTs due to their larger diffusion coefficients.

4. 4. Conclusions

In this study, we have shown that hybrids of DNA and SWCNTs will differentially bind to a conventional AFM imaging substrate (freshly-cleaved mica) in a manner that significantly depends upon the sequence of DNA. By displacing the DNA from the SWCNTs, we have shown that the length distributions of the resulting surfactant-coated

SWCNTs are statistically identical. Spin-coating of these DNA-displaced samples results in larger length distributions that still do not depend on DNA sequence. Therefore, we surmise that the DNA mediates the interactions between the DNA-SWCNT hybrids and the imaging substrate. By quantifying the weight and coverage of DNA molecules on each SWCNT, we find that (GT)₆ binds with a higher density to shorter SWCNTs and that this difference between DNA sequences can explain the observed discrepancies in apparent length distributions of the surface-bound samples. Finally, we propose that for accurate length determinations of SWCNT samples through AFM measurements, DNA-SWCNTs should be transferred to a surfactant wrapping prior to surface adsorption through a spin-coating method.

4. 5. Materials and Methods

DNA-SWCNT Sample Preparation: Single-walled carbon nanotubes produced by the HiPco process (Nanointegris) were used throughout the study. For each dispersion, 1 mg of raw nanotubes was added to 2 mg of desalted (GT)_n (n = 6 or 30) oligonucleotide (Integrated DNA Technologies) in a microcentrifuge tube with 1 mL of 100 mM NaCl (Sigma-Aldrich). The mixtures were then ultrasonicated using a 1/8" tapered microtip (Sonics Vibracell; Sonics & Materials) for 30 min at 40% amplitude, with an average power output of 8 W, in a 0 °C temperature-controlled microcentrifuge tube holder. After sonication, the dispersion was ultracentrifuged (Sorvall Discovery M120 SE) for 30 min at 250,000 xg, and the top 80% of the supernatant was extracted. The concentration was determined with a UV/vis/NIR spectrophotometer (Jasco, Tokyo, Japan), using the extinction coefficient of $A_{910} = 0.02554^{38, 61, 62} \text{ L.mg}^{-1}.\text{cm}^{-1}$. The free DNA was removed using 100

kDa Amicon centrifuge filters (Millipore). For each sample, filtration was repeated 4 times and the pellet was resuspended in 100 mM NaCl in each step. The resultant suspensions were used for concentration measurement and diluted in 100 mM NaCl to reach the final DNA-SWCNT concentration of 20 mg/L and used for further AFM imaging and DNA quantification.

Surfactant Displacement of DNA: The resultant filtered suspensions of DNA-SWCNTs were diluted in 100 mM NaCl+0.1 wt% SDC to give the final DNA-SWCNT concentration of 20 mg/L. These mixtures were kept at 85 °C for 30 min to displace the DNA with SDC.³⁵ The displaced DNA and free SDC were separated from SDC-SWCNTs using 100 kDa Amicon centrifuge filters (one-time filtration). The DNA-displaced SDC-SWCNTs pellet was resuspended in 100 mM NaCl to reach the final concentration of 20 mg/L, and the final suspension was used for AFM imaging.

Quantification of DNA: To quantify the amount of DNA on the SWCNTs, the mixture of the displaced DNA and free SDC (*i.e.* the flow-through from the previous 100 kDa filtration) was filtered two times using a 3 kDa Amicon centrifuge filter to separate the DNA from SDC. The DNA remained in the filter and was resuspended in 100 mM NaCl. The weights of the DNA and SWCNTs (*i.e.* the weight of DNA-displaced SDC-SWCNTs from the previous step) were calculated by the extinction coefficients of $A_{260}=0.027^{60}$ and $A_{910} = 0.02554^{61}$ L.mg⁻¹.cm⁻¹, respectively.

Atomic Force Microscopy: Similar to previous studies,⁶³ the length distribution of the DNA-SWCNT and DNA-displaced SDC-SWCNT samples was measured using atomic force microscopy. Free DNA was removed using a 100 kDa Amicon centrifuge filter (Millipore). A stock solution of DNA-SWCNTs or DNA-displaced SDC-SWCNTs

at 20 mg/L was deposited on AP-mica for 4 min before rinsing with 10 mL of DI water and blowing dry with argon gas. AP-mica was produced by treating freshly-cleaved mica with aminopropyltriethoxysilane (AP) via the vapor deposition method. Additionally, the stock solution of DNA-displaced SDC-SWCNTs was diluted to 10 mg/L and 200 μ L of the sample was spin-coated (Brewer Science CEE-200) onto a cleaned silicon wafer at 2,000 rpm. Excess surfactant was removed from the substrate using DI water. The wafer was pre-cleaned by being placed in an ultrasonic bath (Fisher Scientific 1.9L) in a 50:50 solution of pure ethanol (Sigma-Aldrich) and acetone (Sigma-Aldrich) for 10 minutes before being rinsed and bath sonicated for an additional 10 minutes in DI water. An Asylum-MFP-3D-BIO AFM with Olympus AC240TS probe was used to image in AC mode. The data was captured with 2.93 nm/pixel xy resolution and 15.63 pm z resolution.

Statistical Analysis: Statistical measurements and analyses were performed in OriginPro 2016 using two-sample t-tests under the null hypothesis. Image analysis was performed using Image J software.

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Chapter 5

Enhancing the Thermal Stability of Carbon Nanomaterials with DNA

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5. 1. Summary

Single-walled carbon nanotubes (SWCNTs) have recently been utilized as fillers that reduce the flammability and enhance the strength and thermal conductivity of material composites. Enhancing the thermal stability of SWCNTs is crucial when these materials are applied to high temperature applications. In many instances, SWCNTs are applied to composites with surface coatings that are toxic to living organisms. Alternatively, single-stranded DNA, a naturally occurring biological polymer, has recently been utilized to form singly-dispersed hybrids with SWCNTs as well as suppress their known toxicological effects. These hybrids have shown unrivaled stabilities in both aqueous suspension or as a dried material. Furthermore, DNA has certain documented flame-retardant effects due to the creation of a protective char upon heating in the presence of oxygen. Herein, using various thermogravimetric analytical techniques, we find that single-stranded DNA has a significant flame-retardant effect on the SWCNTs, and effectively enhances their thermal stability. Hybridization with DNA results in the elevation of the thermal decomposition temperature of purified SWCNTs in excess of 200 °C. We translate this finding to other carbon nanomaterials including multi-walled carbon nanotubes (MWCNTs), reduced graphene oxide (RGO) and fullerene (C₆₀), and show similar effects upon complexation with DNA. The rate of thermal decomposition of the SWCNTs was also explored and found to significantly depend upon the sequence of DNA that was used.

5. 2. Introduction

Reduced flammability, defined as a high thermal stability and/or low heat release rate, is a desirable feature of novel advanced materials.¹⁻³ A variety of synthetic flame-

retardant chemicals are incorporated into polymer composites,⁴⁻⁶ and textiles,⁷⁻⁹ to decrease the flammability of manufactured products such as electronics, building, and construction materials, and furnishings. Synthetic flame retardants can be classified into halogenated,¹⁰ (*e.g.* brominated and chlorinated) and non-halogenated¹¹ (primarily phosphorus-containing). Studies in laboratory animals and humans have linked the most widely used flame retardants, polybrominated diphenyl ethers (PBDEs), to thyroid disruption,¹² memory and learning problems,¹³ and reduced fertility.¹⁴ Unfortunately, the synthetic chemicals added to consumer products to meet federal and state flammability standards have been reported to enter waterways,¹⁵ wildlife,¹⁶ and even human breast milk.¹⁷

Deoxyribonucleic acid (DNA) has been proven as an intrinsically intumescent flame retardant,^{18, 19} as it contains the three typical components of an intumescent formulation. First, the phosphate backbone in DNA phosphorylates the substrate and produces a polymeric form of phosphoric acid under flame conditions.¹⁸⁻²⁰ This acid “char” is thermally-insulating and further protects the substrate surface from oxygen and/or flames. Next, the deoxyribose sugars in DNA tend to dehydrate upon heating, form a char, and release water.^{18, 19} Finally, the nitrogen-containing nucleobases give rise to the formation of azo-compounds which are able to further induce a char development in addition to the production of non-combustible gases (notably, N₂, CO₂ and CO).^{18, 19}

Single-walled carbon nanotubes (SWCNTs) functionalized with surfactants,²¹ and amphiphilic polymers,²² have recently been developed and utilized in various disparate fields ranging from targeted anti-cancer drug delivery,²³ to near-infrared optical sensing,²⁴ and biological imaging.²⁵ Of significant interest, SWCNTs exhibit flame-retardant properties,²⁶ extraordinarily high young modulus,^{27, 28} and thermal conductivity,²⁹ which make

them ideal fillers that can improve both flammability,³⁰ and mechanical properties of polymer nanocomposites.^{31, 32} In flame retardant applications, SWCNTs form a structured network that covers the surface of the nanocomposite.²⁶ Upon exposure to flame at elevated temperature, this layer acts as heat shield to slow the thermal degradation of the polymer below the nanotube film.²⁶ Enhancing the thermal stability of SWCNTs is therefore desired to decrease the flammability of such composites. Such SWCNTs with enhanced thermal stability will be advantageous in other high temperature applications, *e.g.* high temperature sensing,³³ and electronics.³⁴

Due to its hydrophobic bases and a hydrophilic backbone, single-stranded DNA has been demonstrated to helically wrap and non-covalently attach to the outside of the SWCNTs by hydrophobic π - π stacking interactions, and enable single-particle dispersion of the SWCNTs in aqueous environments.³⁵ Probe-tip sonication is generally performed to induce enough energy in order to break up initially bundled SWCNTs and individually disperse them in water.³⁶ Considering the mentioned flame-retardant ability of DNA coupled with its unique helical conformation on the SWCNTs side-wall, it can act as an effective protection for SWCNTs against thermal decomposition. DNA-SWCNT hybrids are promising candidates to be utilized as flame retardants in the production of flame-resistant nanocomposites due to unique characteristics of SWCNTs and reports on minimal toxicity of DNA-SWCNT hybrids,^{37, 38} despite the toxicity of convectional flame retardants,¹² raw SWCNTs and surfactant coated SWCNTs.³⁹

Thermogravimetric analysis (TGA) can reliably be used to characterize and elucidate the purity of nanomaterials.⁴⁰ TGA is a straightforward analytical technique that quantifies mass loss when a material is heated in the absence or presence of oxygen. Information

on the thermal decomposition temperature, *i.e.* the temperature of maximum weight loss, thermal decomposition rate in a rapid temperature increase,⁴⁰ and residual mass of the sample are obtained from the decomposition curve (weight vs temperature or time). The residual mass after decomposition could be due to inorganic nanomaterials, residual metal catalysts from synthesis, or impurities within the sample.⁴¹

Moreover, confocal Raman microscopy can be utilized in order to characterize the structural integrity of the SWCNTs after exposure to elevated temperatures. The Raman signal from SWCNTs, particularly the intensity of the G-band (located $\sim 1589\text{ cm}^{-1}$), is dependent only on the amount of sp^2 -hybridized graphitic carbon present in a sample,⁴² and is not affected by noncovalent functionalization. Other signature SWCNT Raman features that can be used to elucidate their structure include the radial breathing mode (RBM, located $\sim 200\text{-}300\text{ cm}^{-1}$) and the D-band ($\sim 1340\text{ cm}^{-1}$), which correlate to nanotube diameter and disorder of sp^2 -hybridization respectively.⁴³

In this report, using a thermogravimetric analytical approach, we examined the thermal decomposition characteristics (thermal decomposition temperature and rate) of DNA functionalized carbon nanomaterials. We find that hybridization of the nanomaterials with DNA increased the thermal decomposition temperature of HiPco SWCNTs in excess of $200\text{ }^{\circ}\text{C}$. We also find that hybridization with DNA significantly increased the thermal decomposition temperature of other carbon nanomaterials such as multi-walled carbon nanotubes (MWCNTs), reduced graphene oxide (RGO), and fullerene (C_{60}). Furthermore, the thermal decomposition rate in an instantaneous temperature increase can be controlled by manipulating the sequence of the DNA used in the construction of the hybrids.

5. 3. Results and Discussion

Thermogravimetric analysis was used to assess the thermal decomposition temperature (maximum weight loss temperature) of DNA-SWCNT hybrids in addition to their constitutive components. Weight percent versus temperature data were acquired at the rate of 20 °C/min for raw HiPco SWCNTs, purified SWCNTs, pure DNA, and DNA-SWCNT hybrids (Figure 5. 1a-c, and S5. 1-S5. 7). In order to delineate the temperature of thermal decomposition, first derivative plots were created (Figure 5. 1d-f). In the case of raw SWCNTs and purified SWCNTs, an abrupt decrease in the mass of the sample occurred at 311 ± 3 °C and 328 ± 2 °C, respectively (Figure 5. 1a, d and S5. 1a-d). The lower thermal decomposition temperature of the raw SWCNTs compared to the purified SWCNTs was presumably due to the fast oxidization of the iron catalyst particles⁴⁴, which accounted for greater than 40% of the total weight of the raw SWCNT sample, and remained stable past 1000 °C. X-ray photon spectroscopy (XPS) was performed on raw HiPco SWCNTs and the residual material after thermal decomposition to quantify the elemental composition of the sample (Figure S5. 1e-f). In the raw HiPco sample, only carbon and oxygen were observed as the atomic percentage of iron/iron oxide would be too low in relation to the SWCNTs to detect (Figure S5. 1e). However, the comparable atomic percentage of the iron to carbon and oxygen in the residual decomposed sample confirms the presence of iron/iron oxide as an oxidizer in the thermal decomposition process (Figure S5. 1f). Since we purify the DNA-SWCNTs from the catalyst material through ultracentrifugation³⁵, purified SWCNTs were used as control throughout the remainder of this study.

Additionally, pure DNA exhibited a continual weight loss over almost the entire examined temperature range. Two temperatures of significant mass loss occurred at $235 \pm$

1 °C and 838 ± 3 °C (Figure 5. 1b and e) which were independent of DNA sequence length (Figure S5. 7). This can be attributed to the fact that DNA starts to decompose near 200 °C, forming significant amounts of char with enhanced thermal stability up to greater than 800 °C¹⁸.

Figure 5. 1. Weight percent versus temperature profiles of (a) purified SWCNTs, (b) pure (GT)15 DNA oligonucleotide, and (c) (GT)15-SWCNT hybrids. The first-derivatives of these respective plots, $d(Wt\%)/dT$, are computed and presented in (d-f).

To interrogate the enhanced thermal stability that DNA imparts on the SWCNTs, the thermal decomposition temperature of SWCNTs functionalized with DNA was compared to that of purified SWCNTs and pure DNA. There were two temperatures of significant weight loss in the DNA-SWCNT decomposition curve (Figure 5. 1c and f), which could be attributed to the DNA and SWCNT decomposition, respectively. The single-stranded DNA sequence (GT)₁₅, when conjugated to SWCNTs, decomposed at 248 ± 2 °C. This represented a marginally elevated decomposition temperature relative to pure (GT)₁₅ DNA not conjugated to SWCNTs. This can be attributed to the known flame-retardant characteristics of the SWCNTs³⁰. A consistent trend was seen in DNA-SWCNT hybrids constructed from other sequences (Figure S5. 8a). The second temperature of interest, representing the presumed SWCNT thermal decomposition temperature, was located at 551 ± 3 °C. Compared to the purified SWCNTs, this represented a substantial increase of 250 °C ($p < 0.001$) which was fairly insensitive to DNA sequence (Figure S5. 8b). Our working hypothesis is that the DNA, which non-covalently wraps individual SWCNTs, expands upon heating, thus encapsulating and thereby protecting the SWCNT from decomposition. Additionally, we acquired the weight versus temperature profiles for DNA-SWCNTs at the heating rate of 10 °C/min (Figure S5. 5e-f). Although there is not any substantial change in shape (i.e. appearance of new peaks) in the profile, we observe a split peak for the SWCNT material (major peak at 585 ± 5 °C, minor peak at 524 ± 6 °C), suggesting two populations of SWCNT material. Interestingly, the average of these two peaks results in a value that is not significantly different from the second peak of DNA-SWCNTs at the 20 °C/min heating ramp (554 ± 3 °C) (Figure S5. 5g).

In order to prove the formation of DNA char on the SWCNTs, transmission electron microscopy (TEM) was performed on (GT)₁₅-SWCNT samples exposed to various temperatures. Figure 5. 2a-c illustrate electron micrographs of the dried (GT)₁₅-SWCNT hybrids at room temperature, and after exposure to 400 and 700 °C, respectively.

Figure 5. 2. TEM images of (GT)₁₅-SWCNTs exposed to (a) room temperature, (b) 400°C, or (c) 700 °C for 30 minutes. All samples imaged at room temperature. The red arrows point to the SWCNTs.

Individual SWCNTs are observed in the room temperature sample (red arrows in Figure 5. 2a), consistent with previous reports.⁴⁴ Interestingly, after exposure to 400 °C, SWCNTs appeared embedded in an electron dense structure, (red arrows in Figure 5. 2b)

presumably the char produced by the DNA (Figure S5. 9). Finally, the image of (GT)₁₅-SWCNT hybrids after exposure to 700 °C indicated a different morphology altogether (Figure 5. 2c), with no resemblance of a tube-like material. We use these TEM images as evidence that a thick char-like layer is forming on the SWCNTs and increasing their thermal stability.

To confirm that the structural integrity of the SWCNTs is preserved after exposure to elevated temperatures, we performed confocal Raman microscopy on purified SWCNTs and (GT)₁₅-SWCNTs dried on silicon wafers. Initially, both samples produced intense G-band signals at room temperature (Figure 5. 3a and c). However, a 30-minute exposure to 400 °C resulted in significantly reduced G-band intensity and a loss of other characteristic Raman features of purified SWCNTs (Figure 5. 3a-b), indicating that the SWCNTs were mostly decomposed or significantly damaged at the elevated temperature. Conversely, the G-band intensity maps of SWCNTs functionalized with DNA displayed minimal differences before and after exposure to 400 °C, although the slight decrease in intensity suggests that a small portion of SWCNTs had decomposed (Figure 5. 3c-d). As expected from TGA profiles, both samples were fully decomposed at 700 °C (Figure S5. 10), resulting in spectra containing a single peak corresponding to the silicon wafer (528 cm⁻¹). Regarding the RBM spectral feature, (GT)₁₅-SWCNTs at room temperature were shifted ~6 cm⁻¹ from the RBM value of purified SWCNTs and did not have the same features below 240 cm⁻¹ due to presence of a DNA-wrapping (Figure 5. 3e).⁴⁵⁻⁴⁷ Interestingly, exposure of (GT)₁₅-SWCNTs to 400 °C induced a 5 cm⁻¹ shift from its original position back to the value of the purified SWCNT signal.

Figure 5.3. Brightfield images and G-band confocal Raman intensity maps of (a) purified SWCNTs and (c) (GT)₁₅-SWCNTs dried on silicon wafers before and after a 30-minute exposure to 400 °C. The average spectra of SWCNT-containing pixels in confocal Raman area scans of (a) and (c) are shown in (b) and (d) respectively. Average Raman spectra zoomed-in to show detail in (e) normalized RBM, (f) D-band, and (g) G-band of (GT)₁₅-SWCNTs at room temperature and after a 30-minute exposure to 400 °C. Scale Bar = 100 μm .

These data suggest that most of the DNA had decomposed, leaving behind

SWCNTs that were structurally similar to the purified samples. Comparison of average Raman spectra before and after heating (GT)₁₅-SWCNTs to 400 °C (Figure 5. 3d) showed an increase in the overall intensity of the D-band and a local increase at 1560 cm⁻¹ of the G-band (Figure 5. 3f-g). These changes are characteristic of increasing amounts of amorphous carbon,^{48, 49} which when taken with the decreased DNA content observed from the RBM signals, confirm the formation of a protective char around the SWCNTs.

To demonstrate a potential flame-retardancy effect of DNA on other carbon nanomaterials, we functionalized MWCNTs, RGO and C₆₀ with the same (GT)₁₅ DNA sequence and quantified their thermal decomposition temperature (the temperature of the second significant mass loss in the first-derivative weight versus temperature profiles). Similar to SWCNTs, DNA wraps around MWCNTs and C₆₀ by hydrophobic π - π stacking interactions,^{50, 51} while it randomly orients in between the RGO sheets through π - π stacking interactions.^{52, 53} In all these cases, DNA causes single particle exfoliation of these carbon nanomaterials in water. In a similar fashion, weight percent versus temperature and first derivative plots were created for all of the materials (Figure 5. 4a-d and S5. 11-S5. 13). Comparing all DNA-carbon nanomaterials with the appropriate controls, the bar graph in Figure 5. 4e indicates substantial increases in the thermal decomposition temperature of all examined carbon nanomaterials in the order SWCNTs > C₆₀ > RGO > MWCNTs.

Figure 5. 4. First-derivative weight percent versus temperature plots for (GT)₁₅ DNA functionalized- (a) SWCNTs, (b) MWCNTs, (c) RGO, and (d) C₆₀. (e) Bar graph to compare the increase in the thermal decomposition temperature of the carbon nanomaterials functionalized with DNA to that of raw/purified nanomaterials. TGA was repeated three times (n=3) for each sample. Two-sample t-tests were performed (**, $P < 0.01$, ***, $P < 0.001$).

In addition to the thermal decomposition temperature, the decomposition rate in an instantaneous temperature increase is another important parameter associated with thermal stability. To compare the raw SWCNT thermal decomposition rate to that of the DNA-

SWCNT hybrids, we performed a weight percent versus time assay when the material was subjected to a rapid temperature increase from 400 to 700 °C (Figure 5. 5). It is known that the compactness of the DNA corona on the SWCNTs can be modulated by DNA base type.⁵⁴ Therefore, in addition to the (GT)₁₅ DNA sequence, we functionalized the SWCNTs with cytosine-containing sequences (CT)₁₅ and C₃₀, as cytosine-rich sequences display a significantly reduced desorption from the SWCNTs compared to other sequences and form more compact packing structures on the SWCNTs, resulting in more DNA coverage on the SWCNTs.⁵⁴ Here, our hypothesis is that more DNA bound to the SWCNT should correlate to slower thermal decomposition rates for DNA-SWCNT hybrids. Indeed, the increase in the cytosine content of the sequence significantly decreased the apparent decomposition rates (increased the decomposition time constants) of the resultant DNA-SWCNT hybrids (Figure 5. 5a-b). Figure S5. 14 and S5. 15 demonstrate the weight percent versus time profiles of (GT)₁₅-, (CT)₁₅-, and (C)₃₀-SWCNT hybrids and their appropriate controls. The temperature was first set to 400 °C and held constant for 30 minutes to allow for DNA char formation. After 30 minutes, the temperature was rapidly raised to 700 °C and kept constant for 30 minutes. Figure S5. 14b-d demonstrate that there is no significant mass loss after the first rapid temperature increase where the temperature is held constant at 400 °C for 30 minutes. This confirms the DNA char assisted protection for SWCNTs against decomposition at 400 °C. We fitted the decay processes of DNA-SWCNTs in the second step (rapid temperature increase from 400 to 700 °C) to a single exponential (Figure 5. 5a and S5. 16) and report a quantified time constant for each sample (Table S5. 1).

Figure 5. 5. (a) Weight percent versus time profiles for purified SWCNTs, and DNA-SWCNTs composed of (GT)₁₅, (CT)₁₅, or C₃₀ DNA sequences, when the temperature is instantaneously increased from 400 to 700 °C. The data are fitted to a single exponential decay and the resultant curve is plotted as a black dotted line. (b) Bar graph to compare the thermal decomposition rate (1/thermal decomposition time constant) of raw SWCNTs and DNA-SWCNTs made from (GT)₁₅, (CT)₁₅, or C₃₀ DNA sequences. TGA was repeated three times (n=3) for each sample. A two-sample t-test was performed (*, $p < 0.05$, **, $p < 0.01$).

Due to the poor fit of the data in the case of purified SWCNTs (Figure S5. 16a), which may be attributed to limitations in the instrument's ability to instantaneously raise the temperature to the desired set-point, the time constant for this sample was calculated from its definition (i.e. the time required for a system to reach $1/e$ ($\sim 36.8\%$) of its initial value). Comparing all DNA sequences to the SWCNT control, Figure 5. 5b shows that DNA-SWCNTs decompose in the rate order of $C_{30} < (CT)_{15} < (GT)_{15}$. Therefore, an increase in the relative cytosine content in the DNA sequence significantly enhances the thermal stability characteristics of the SWCNTs.

5. 4. Conclusions

In this work, we examined the thermal decomposition temperature and rate of DNA functionalized carbon nanomaterials using thermogravimetric analysis. We find that functionalization with DNA significantly increases the thermal decomposition temperature of all examined carbon nanomaterials in the order $SWCNTs > C_{60} > RGO > MWCNTs$. We attribute these increases in decomposition temperature to the fact that DNA, an intumescent molecular entity that increases in volume upon heating, shields the hybridized carbon nanomaterials from flame and elevated temperatures (Figure 5. 6). Moreover, we control the decomposition rate of the SWCNTs by manipulating the sequence of the DNA and show that a higher cytosine content corresponds to a higher degree of flame retardancy. Enhancing the thermal decomposition properties of carbon nanomaterials with non-toxic flame retardants enables the realization of environmentally friendly high temperature sensing and electronics applications.

Figure 5. 6. Illustration of DNA char assisted protection for SWCNTs against thermal decomposition. SWCNTs without DNA coverage decompose at high temperatures while SWCNTs noncovalently wrapped with DNA remain stable at high temperatures.

5. 5. Materials and Methods

DNA Assisted Dispersion of SWCNTs: SWCNTs produced by the HiPco process (Nanointegris) were used throughout the study. SWCNTs were dispersed with single-stranded DNA oligonucleotides in 1 mL of 100 mM NaCl (Sigma-Aldrich), by adding 1 mg of raw nanotubes to 2 mg of desalted (GT)_n ($n = 3, 6, 9, 12, 15, \text{ or } 30$), (CT)₁₅, or C₃₀ oligonucleotide (Integrated DNA Technologies) in a microcentrifuge tube. The mixtures were ultrasonicated using a 1/8" tapered microtip (Sonics Vibracell; Sonics & Materials) for 30 minutes at 40% amplitude, with an average power output of 8 W, in a 0 °C temperature-controlled microcentrifuge tube holder. After sonication, the dispersion was ultracentrifuged (Sorvall Discovery M120 SE) for 30 minutes at 250,000 g, and the top 80% of the supernatant was extracted. The concentration was determined with a UV/vis/NIR spectrophotometer (Jasco, Tokyo, Japan), using the extinction coefficient of $A_{910} = 0.02554^{55}$ L.mg⁻¹.cm⁻¹. The free DNA was removed using 100 kDa Amicon centrifuge filters (Millipore). For each sample, filtration was repeated 4 times, and the DNA-SWCNTs pellet was resuspended in DI water in each step including the final step.

Preparation of the Purified SWCNTs from the (GT)₁₅-SWCNTs: The free DNA was first removed following the protocol reported in the previous section. The DNA bound to the SWCNTs was displaced with a surfactant (sodium deoxycholate (SDC)) according to the procedure reported in our previous study.⁵⁶ The SDC in the resultant SDC-SWCNT samples was displaced and removed using multiple Amicon filtration steps (5-8 times). The purified SWCNT aggregates were then collected for further analysis.

DNA Assisted Dispersion of MWCNTs, RGO, and C₆₀: MWCNTs (Alfa Aesar), RGO (Graphene Supermarket), and C₆₀ (Alfa Aesar) were dispersed with (GT)₁₅ DNA sequence in 1 mL of 100 mM NaCl, by adding 1 mg of raw nanomaterials to 2 mg of desalted DNA in a microcentrifuge tube. The mixtures were ultrasonicated using a 1/8" tapered microtip for 30 minutes (Only the C₆₀ solution was ultrasonicated for 90 minutes) at 40% amplitude, with an average power output of 8 W, in a 0 °C temperature-controlled microcentrifuge tube holder. The free DNA was removed using 100 kDa Amicon centrifuge filters (Millipore), and the DNA functionalized carbon nanomaterials pellet was resuspended in DI water.

Thermogravimetric Analysis (TGA): 500 μ L of samples of pure desalted (GT)_n DNA, raw/purified carbon nanomaterials, or DNA functionalized carbon nanomaterials with free DNA and salt removed were added to Platinum pans and the water of the samples evaporated. The pans were placed in a TGA instrument (TA Instruments, Q500) and the instrument was programmed to perform different functions. To quantify the thermal decomposition temperatures of the samples, the temperature was increased by 20 °C /min or 10 °C /min from room temperature to 1000 °C, through a ramp function. To investigate the thermal decomposition rates (time constants) of the DNA-SWCNTs, the temperature was

jumped to 400 °C and then 700 °C, and held constant at each of the temperatures for 30 minutes to make sure that the DNA decomposes at the first step and SWCNTs decompose at the second step.

Transmission Electron Microscopy (TEM): (GT)₁₅-SWCNT samples (free DNA and salt removed by filtration) at room temperature were imaged on carbon-coated TEM grids (Electron Microscopy Sciences) by depositing a 5 μ L drop on the center of the grid, and letting it dry in room temperature. Additionally, to perform the TEM of (GT)₁₅-SWCNT samples decomposed at 400°C and 700°C, a 5 μ L drop of the sample (free DNA and salt removed by filtration) was placed on the center of PELCO Silicon Nitride Support grids (Ted Pella), and dried at room temperature. The grids were then placed in TGA pans and the temperature was jumped to either 400°C or 700°C, and held constant at each temperature for 30 minutes. The grids were then used for TEM imaging (JEOL JEM-2100F) operating at 200 kV.

Confocal Raman Microscopy: Purified SWCNTs or (GT)₁₅-SWCNTs (free DNA and salt removed by filtration) were deposited on silicon carbide wafers and the water was evaporated. The samples were imaged using a WITec alpha300 R confocal Raman microscope with a 532 nm excitation laser and 10x objective, and Raman spectra were obtained in 10 μ m intervals across the image area. Each sample was imaged directly after drying and again after incubation at either 400°C or 700°C for 30 minutes. The SWCNT-containing Raman spectra were determined by the presence of the G-band, and all SWCNT-containing pixels were averaged to obtain a single Raman spectrum for each condition.

Statistical Analysis: Statistical measurements and analyses were performed in OriginPro 2016 using two-sample t- tests under the null hypothesis.

5. 6. Acknowledgments

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5. 7. References

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Chapter 6

A Wearable Optical Microfibrous Biomaterial with Encapsulated Nanosensors Enables Wireless Monitoring of Oxidative Stress

(Under Review in Advanced Functional Materials)

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6. 1. Summary

In an effort to facilitate personalized medical approaches, the continuous and non-invasive monitoring of biochemical information using wearable technologies can enable a detailed understanding of an individual's physiology. Reactive oxygen species (ROS) are a class of oxygen-containing free radicals which function in a wide range of biological processes. In wound healing applications, the continuous monitoring of ROS through a wearable diagnostics platform is essential for the prevention of chronicity and pathogenic infection. Here, a versatile one-step procedure is utilized to fabricate optical core-shell microfibrillar textiles incorporating single-walled carbon nanotubes (SWCNTs) for the real-time optical monitoring of hydrogen peroxide concentrations in wounds. The environmentally sensitive and non-photobleachable fluorescence of SWCNTs enables continuous analyte monitoring without a decay in signal over time. The existence of multiple chiralities of SWCNTs emitting near-infrared fluorescence with narrow bandwidths allows a ratiometric signal readout invariant to the excitation source distance and exposure time. The individual fibers encapsulate the SWCNT nanosensors for at least 21 days without apparent loss in structural integrity. Moreover, the microfibrillar textiles can be utilized to spatially resolve peroxide concentrations on a wound surface using a camera and can be integrated into commercial wound bandages without being altered or losing their optical properties.

6. 2. Introduction

Reactive oxygen species (ROS) are continuously generated and consumed in all eukaryotic and prokaryotic cells as a consequence of aerobic life.^{1,2} In this biological context, ROS primarily function to preferentially react with specific atoms of biomolecules

involved in a wide range of physiological processes.¹ ROS play a crucial role in biological signaling including the inhibition or activation of proteins, subsequent promotion or suppression of inflammation, immunity, and carcinogenesis.^{1,3-6} Oxidative stress can occur if the ROS-induced signal is too strong, if it persists for too long, or if it occurs at the wrong time or place.^{1,3,5,7} As a key example, wound healing is one of the most dynamic biological processes involving ROS-linked cellular signaling throughout the entire mechanism.⁸⁻¹¹ Additionally, basal concentrations of ROS aid in the fight against invading microorganisms into open wounds.^{4,11,12} The excessive and uncontrolled production of ROS contributes to the sustaining and deregulation of inflammation processes, which play a central role in the pathogenesis of chronic non-healing wounds.¹³⁻¹⁷ Physiologically, hydrogen peroxide (referred herein as peroxide) and superoxide function as intracellular ROS messengers stimulating key phases of wound healing including cell recruitment, production of cytokines, and angiogenesis.^{16,18,19} Of note, peroxide acts as the principal secondary messenger in wound healing and is present at low concentrations (100–250 μM) in normal wounds.^{8,11,19,20} Increased peroxide concentration is a biomarker for inflammation and chronicity in which biofilm-forming pathogens can grow significantly faster than acute wounds.^{15,17,20} Interestingly, strictly controlling the ROS levels through antioxidants has recently been shown to improve inflammatory skin conditions and wound healing process in diabetic and hypoxic environments.²¹

Due to the short half-lives of ROS, the direct detection and quantification of their concentrations are often difficult in the laboratory and in patients.²⁰ Although multiple classes of sensors and spectrophotometric assays have been developed to monitor various types of ROS, the current methods are limited in their capabilities. Spectrophotometric

methods,²² such as total antioxidant capacity assays (TAC),²³ and gel electrophoresis¹⁴ have been utilized to indirectly determine the oxidation products of lipids, proteins and DNA, but these are not capable of real-time monitoring in the wound site.²⁰ Various label-free electrochemical biosensors have been developed to accurately quantify the ROS concentrations by immediately converting the chemical information to an electrical signal.^{19, 24, 25} The main drawback of electrochemical techniques is the requirement to incorporate electrodes into different biomaterials and wireless platforms. Moreover, the need to utilize an electrochemical signal transducer restricts the application of the current sensors on wounds in different organs of the body. Fluorescent nanoparticles²⁶⁻²⁸ and genetically encoded fluorescent molecules^{8, 29-31} highly selective for peroxide have been created to study the redox events in mice, zebrafish, and cells, but these assays cannot be utilized for real-time monitoring in clinical applications. Therefore, developing a point-of-care diagnostic technology for the real-time monitoring of ROS concentrations in wound sites is essential to prevent chronicity and infection, and to deliver accurate amounts of antioxidants and antibiotics to the wounds.

Single-walled carbon nanotubes (SWCNTs) with engineered wrappings have recently been developed and utilized in various disparate fields ranging from additives that strengthen material composites^{32, 33} to biomedical applications including near-infrared (NIR) optical biosensing,³⁴⁻³⁶ and biological imaging.^{37, 38} The electronic band gap energies of SWCNTs are dependent on their chiral identity, denoted by integers (n,m), and vary based on diameter and rollup angle, resulting in various semiconducting species which exhibit a distinct narrow-bandwidth photoluminescence in the second NIR window.³⁹ The SWCNT photoluminescence responds to their local environment,⁴⁰ resulting in shifts in

emission wavelengths⁴⁰⁻⁴³ and/or variations in intensity.^{44, 45} Certain amphiphilic polymers such as short single-stranded deoxyribonucleic acids (ssDNA),⁴¹ Phospholipid-Polyethylene glycol (PL-PEG),⁴⁶ and synthetic polymers³⁴ have all been shown to effectively solubilize SWCNTs, imparting enhanced biocompatibility and desirable fluorescence properties. The resultant hybrid nanomaterials have been optimized for the detection of a wide range of analytes *in vivo* and *in vitro* including neurotransmitters,⁴⁵ lipids,³⁵ and proteins.⁴³ SWCNT-based optical nanosensors have also recently been developed for real-time spatial and temporal monitoring of ROS in various plant species as a biomarker for plant health.^{44, 47} Moreover, ratiometric SWCNT-based optical sensors have enabled the real-time monitoring of ROS in plants, allowing an absolute calibration independent of overall intensity.⁴⁸ The current ratiometric sensing approaches based on SWCNTs require separation of at least two highly pure single chiralities, wrapped in two different polymers, where one polymer-chirality pair is sensitive to the local environment and the other pair does not spectrally respond to the variations in the local environment and acts as a reference.⁴⁸

Although the ssDNA- and polymer-wrapped SWCNT nanosensors have attracted significant interest in the past decade for biosensing applications *in vivo* and *in vitro*, the integration of these biosensors into other bulk biomaterial platforms has been a challenge as their NIR fluorescence is remarkably sensitive to the chemistry of their local environment and can be suppressed by other components in the biomaterial preparation processes.^{34, 40} Moreover, due to the hydrophilicity of these nanosensors, it is unfavorable to engage them in any process involving organic solvents as they form bulk aggregates in hydrophobic environments.

With revolutionary advances in nanotechnology and biomaterials in recent years, an extensive range of smart wound care biomaterials have been developed that enable localized delivery of drugs on the wound site^{49, 50} and real-time monitoring of the wound microenvironment.^{51, 52} Electrospun microfibers are one of the novel classes of wound dressings as they mimic the chemical and mechanical environment of the 3D extracellular matrix.^{53, 54} Microfiber-based wound dressings have been designed to enhance cell migration,⁵⁵ prevent inflammation and infection,⁵⁶ and inhibit scar formation on wounds.⁵⁴ Herein, we utilized a one-step co-axial electrospinning process to fabricate wearable microfibrous textiles incorporating peroxide-sensing SWCNTs. The electrospun fibers feature a core-shell morphology in which the SWCNTs are encapsulated inside of a polymer shell that is soluble in an organic solvent. We chose polycaprolactone (PCL) as the shell material as it is an FDA-approved polymer which has been extensively studied for tissue engineering and wound healing applications.⁵⁷⁻⁵⁹ Our wearable optical platform was able to wirelessly and reversibly detect peroxide in a physiologically-relevant range for wounds (1-250 μM). The ratiometric characteristic of the NIR fluorescence sensor facilitates *in vivo* and clinical applications as it transduces an absolute signal that is not dependent on excitation source distance nor exposure time. Utilizing confocal Raman microscopy, we found that the SWCNT nanosensors stay encapsulated within the individual fibers for up to at least 21 days, indicating that the long-term identity of the nanosensing platform is maintained. We also indicate the potential of our optical textiles for spatially resolving the peroxide concentration on the surface of a wound using hyperspectral fluorescence microscopy. Finally, we attached our microfibrous platform to a conventional wound bandage and demonstrated the feasibility of *in situ* measurements of peroxide in wounds.

6. 3. Results and Discussion

6. 3. 1. Preparation and Characterization of Optical Microfibers

We prepared aqueously-dispersed ssDNA-SWCNT nanosensors by probe-tip sonicating HiPco SWCNTs in the presence of single-stranded (GT)₁₅ DNA (Figure 6. 1a). The (GT)₁₅ sequence was selected as SWCNT-based nanosensors with this sequence of DNA have been utilized in live cells and plants for the real-time and selective monitoring of peroxide, in contrast to other signaling molecules including nitric oxide (NO), super oxide ([O₂]^{•-}), singlet oxygen (¹O₂) and hydroxyl radical ([OH][•]).^{27, 44, 48} Following sonication, the sample was ultracentrifuged to remove bundles of undispersed SWCNTs as well as residual catalyst particles to produce an ink-like solution with strong NIR absorbance and fluorescence spectra (Figure S6. 1). With colloiddally stable nanosensors, we employed a core-shell electrospinning procedure to encapsulate the hydrophilic ssDNA-SWCNTs along with poly(ethylene oxide) (PEO) into the polymer polycaprolactone (PCL), that is soluble in an organic solvent (Figure 6. 1b). Briefly, the shell and core are extruded from a two-compartment spinneret, and once injected, form a core-shell pendant droplet as the result of surface tension.⁵⁴ A high voltage is applied to the droplet that produces a two-compartment Taylor cone as well as a constant electric field between the spinneret and a metal grounded collector.⁵⁴ The electrical force significantly elongates the two components of the Taylor cone until they turn into microfibers.⁵⁴ After rapid solvent evaporation, the immiscibility of the core and shell causes complete encapsulation of the core within the shell.⁶⁰ In this process, the hydrophilic ssDNA-SWCNT nanosensors are protected against a prolonged interaction with an organic solvent. Additionally, the intrinsic NIR fluorescence of

the nanosensors is maintained as the process does not introduce any other chemicals such as crosslinking agents. There are a number of physical parameters involved in the electrospinning process which control the reproducibility and homogeneity of the final samples. We have optimized the flow rates of the polymers, rotation rate of the collector, and the distance between the needle and collector to achieve a stable electrospinning jet (data not shown).

Figure 6. 1. Fabrication of optical microfibers. (a) Nanosensor preparation by probe-tip sonicating SWCNTs in the presence of ssDNA followed by ultracentrifugation of the resultant dispersion. (b) Core-shell electrospinning setup for fabrication of the optical microfibrinous textiles. The two syringes containing the core and shell polymer solutions are connected to the inlets of a custom core-shell needle. Once the polymer solutions are injected out, a core-shell pendant droplet is formed. A high-voltage supply is connected to the tip of the needle and electrifies the droplet, forms a Taylor cone, and eventually elongates the cone until microfibers are created. The resultant fibers are collected onto a rotating metal grounded collector.

To optimize the morphology of the fibers and aggregation state of the nanosensors, we have tuned the applied voltage during the electrospinning process. Figure 6. 2 demonstrates NIR broadband fluorescence images (900-1400 nm) and scanning electron microscopy (SEM) images of fibers produced with three different voltages.

Figure 6. 2. NIR broadband fluorescence images of microfibers produced with the applied voltages of (a) 12 kV, (b) 14 kV, and (c) 16 kV. The microfibers were illuminated with a 730 nm laser and images were acquired with a 2D InGaAs array detector in the wavelength range 900-1400 nm. SEM images of the fibers fabricated with applied voltages of (d) 12 kV, (e) 14 kV, and (f) 16 kV.

The applied voltage of 12 kV did not provide a high enough rate of elongation, and as a result, SWCNT aggregates appeared in the NIR fluorescence images (Figure 6. 2a and S6. 2). When the applied voltage was 16 kV, occasional aggregates again emerged along the fibers (Figure 6. 2c and S6. 4), presumably due to incomplete formation of the Taylor

cone. An applied voltage of 14 kV produced a homogenous fiber morphology with no significant spatially localized aggregations (Figure 6. 2b and S6. 3). The SEM images of the fibers produced with the three voltages revealed two subsets of fibers with diameters of more than $\sim 1 \mu\text{m}$ or less than $\sim 100 \text{ nm}$ (Figure 6. 2d-f and S6. 5). By visually comparing the SEM and NIR fluorescence images, we observe that the diameter range of the NIR optical fibers is identical to the micron-size fibers in the SEM images (Figure S6. 2-S6. 5). Thus, although we have produced a matrix of micro- and nanofibers, we acknowledge the fact that the optically-active fibers have sizes of more than $1 \mu\text{m}$.

We employed confocal Raman microscopy to confirm the core-shell morphology and assess the complete encapsulation of nanosensors within the individual fibers. Confocal Raman microscopy is a powerful technique used to analyze multicomponent material samples, in which the unique Raman spectrum of each component can be identified and spatially resolved. SWCNTs exhibit distinct Raman signatures such as the G-band (1589 cm^{-1}), which proportionately scales with increasing amounts of graphitic carbon (i.e. SWCNT concentration),^{61, 62} and the radial breathing mode ($150 - 350 \text{ cm}^{-1}$), which can identify the chiral composition of a SWCNT mixture.^{63, 64} Figure 6. 3a displays the bright-field and G-band intensity overlay of a single as-produced fiber. A k-means clustering analysis was applied to the entire dataset, in which the spectrum from each pixel was partitioned into one of 4 clusters ($k = 4$) based on the location and intensity of individual peaks, creating 4 average spectra which best represented all regions, including the background, from the Raman area scan (Figure 6. 3b and c).⁶⁵ Based on the clustering analysis, each individual fiber was categorized to three areas: core, intermediate, and shell (background constituted the fourth cluster). The average Raman spectrum of the core area corroborated

previous reports of SWCNT Raman spectra (Figure 6. 3c).^{32, 41} The average Raman spectrum of the shell area predominantly matched with the spectrum from the PCL polymer (Figure S6. 6a) with two additional peaks at ~ 1589 and 240 cm^{-1} , which can be correlated to small quantities of SWCNTs. However, it is worth noting that SWCNTs benefit from signal enhancement due to resonance Raman scattering,⁶⁶ and thus the intensity of their peaks in the shell clusters could over-represent their actual quantity with respect to PCL. Interestingly, the average Raman spectrum from the intermediate area features spectral characteristics from both SWCNTs and PCL polymer, indicating an area of heterogeneity at the core-shell interface. These results demonstrate that the highest density of the nanosensors reside in the core area of the fibers, while their Raman signal diminishes in the outward radial direction and the main component becomes the polymer shell. Although the spectral characteristics of the PEO polymer are not apparent in the Raman spectra of the three areas, they are presumably dominated by the enhanced Raman signal of the SWCNTs. Moreover, the Raman spectra of the PCL and PEO polymers show minimal overlap with the G-band and RBM peaks from SWCNTs (Figure S6. 6a and b), indicating that the identified SWCNT signal contains no contribution from the other nanofiber components.

To assess the ability of individual fibers to preserve the nanosensors over time, we soaked the fiber samples in phosphate buffered saline (PBS) solution and performed confocal Raman microscopy at different timepoints. Figure 6. 3d and S6. 7 indicate that the SWCNTs remain encapsulated within individual fibers after 7 days, without forming noticeable aggregates or any deformation in the fiber structure. Moreover, k-means clustering analysis identified the same spectral characteristics for the three identified areas (core,

intermediate and shell) at different time points, further demonstrating that the fibers retain their entire structural integrity in an aqueous environment (Figure 6. 3e-f, and S6. 7b, c, e, f). We acknowledge that the ratios between the peaks in Raman spectra of particularly the shell vary among different time points, but this can be attributed to the slight heterogeneity among individual fibers as we have not imaged the same fiber at different time points.

Figure 6.3. Confocal Raman microscopy of dry fibers and fibers soaked in PBS for 7 days. (a) and (d) The representative overlay of G-band intensity and brightfield images of dry fibers and fibers soaked in PBS for 7 days, respectively. (b) and (e) k-means clustering analysis of all spectra in each area scan, where $k = 4$ clusters (background cluster omitted from figure). (c) and (f) The average Raman spectra obtained from each cluster of (b) and (e).

To quantify the amount of the released nanosensors from a bulk fibrous matrix, the microfibrinous textiles with thickness of ~ 0.7 mm were cut to 1 square inch pieces and soaked in PBS. We collected the PBS solutions over time for up to 21 days and acquired

their Raman spectra (Figure S6. 8). Comparing the Raman spectra of the released nanosensors with that of three standard samples with known concentrations (0.01, 0.1, 1 mg L⁻¹), the lack of G-band signal indicates that the released nanosensors fall within the noise range of our Raman spectrometer. We conclude that over the course of at least 21 days, a negligible amount of the nanosensors are released from the fibers.

6. 3. 2. Ratiometric Peroxide Detection Using Optical Microfibrous Textiles

The HiPco SWCNTs contain multiple chiralities emitting NIR fluorescence in the range of 900-1400 nm (Figure S6. 1b).³⁹ As both chirality and DNA sequence determine the spectral responses of SWCNTs to their local environment,⁴¹ we hypothesized that certain chiralities of SWCNTs within the HiPco sample would differentially respond to hydrogen peroxide due to their differing bandgap energies.⁶⁷ We first acquired NIR hyperspectral fluorescence images of the fluorescent fibers containing (GT)₁₅-SWCNTs (Movie S6. 1) using the optical setup illustrated in Figure 6. 4a. Figure 6. 4c-e indicate the fluorescence intensity of three different chiralities in the same fiber sample, i.e. the (9,4), (8,6), and (8,7)-SWCNT chiralities.⁴¹ To test the environmental sensitivity of the optical fibers to peroxide, we exposed the bulk microfibrous samples (area: 0.5 mm², thickness: ~0.7 mm) to various concentrations of peroxide and acquired fluorescence spectra after 24 hours, utilizing a probe NIR fluorescence spectrometer (Figure 6. 4b), which enabled the resolution of the three mentioned chiralities.

Figure 6.4. (a) The schematic of the optical setup utilized to obtain the hyperspectral NIR fluorescence intensity images from the microfibers. (b) The schematic of the probe NIR fluorescence spectrometer utilized to obtain the fluorescence spectra from bulk microfibrous samples. (c) (9,4), (d) (8,6) and (e) (8,7) chirality intensity images, obtained using the system shown in part a. (f) The fluorescent spectra of the microfibrous samples exposed to various peroxide concentrations, obtained from the system shown in part b. (g) The fluorescent spectra shown in part d were normalized by their max intensity.

In agreement with previous studies,^{44,47,48} Figure 6. 4f and S6. 9 reveal that the three chiralities quench upon exposure to peroxide, however, the extent of quenching varies significantly among the chiralities. By normalizing each plot by its maximum intensity (i.e. the intensity of the (9,4) chirality), we observe that the normalized (8,6) and (8,7) peaks monotonically intensify with increasing peroxide concentration, illustrating that a ratiometric signal can be obtained for peroxide detection (Figure 6. 4g). We selected the (8,7) / (9,4) intensity ratio to further calibrate a biosensor for peroxide detection as it appears to be more sensitive to peroxide concentrations compared to the (8,6) / (9,4) ratio.

6. 3. 3. Calibrating the Microfibrous Textiles for Peroxide Detection

To obtain a calibration curve for aqueous peroxide detection, we exposed the initially dry microfibrous samples to various concentrations of peroxide ranging from 1 μM to 5 mM and monitored the samples over time for up to 72 hours. Figure 6. 5a demonstrates that all samples produced the same initial ratiometric signal, clarifying the reproducibility of our method for fabricating bulk samples of optical fibers encapsulating nanosensors. After 24 hours, a concentration-dependent ratiometric signal was obtained from the samples. While 0 to 5 μM peroxide gave no significant change in the ratiometric signal, it monotonically increased with peroxide concentration in the range of 5 μM to 5 mM and could be fit to a linear function with $R^2 = 0.99$ on a log-log scale of signal vs. concentration (Figure 6. 5a and Table S6. 1). When examined at 48 and 72 hours after the addition of peroxide, the ratiometric signal systematically increases while maintaining the trend in the calibration curve. The data can still be fit to a linear function in the range of 5 μM to 5 mM. To explain the time dependency of the calibration curves, we propose that the

noncovalently wrapped DNA adopts more compact conformations on the SWCNT surfaces over time as they progressively interact with ions, mainly sodium, in the PBS.⁶⁸ This rearrangement alters the fluorescence intensity in a chirality-dependent fashion, and thus the ratio of the peaks over time, regardless of peroxide concentration.⁶⁸ Moreover, the diffusion of the peroxide molecules through the pores in the 3D matrix and through the free spaces in between the polymer chains on the shell is a time-dependent process, so it will result in further fluorescence quenching over time. These two phenomena governing the temporal dependence of the peroxide calibration curve are depicted in Figure 6. 5b.

Figure 6. 5. (a) Calibration curve showing the ratiometric signal, i.e. (8,7) intensity divided by (9,4) intensity, as a function of peroxide concentration at three different time points. Mean values were obtained by adding each peroxide concentration to three different samples ($n = 3$), and error bars represent the standard deviation. (b) Two phenomena governing the temporal dependence of the peroxide calibration curve; (I) The diffusion of peroxide molecules through the 3D matrix of fibers and through the shell to reach to the ssDNA-SWCNTs in the core of the individual fibers. (II) Due to the ionic strength of the surrounding environment, the ssDNA on the surface of nanotubes undergoes a conformational change over time and forms a more compact wrapping around the nanotubes. As a result of these two phenomena, the NIR fluorescence of SWCNTs and the ratiometric signal are altered over time.

Therefore, in order to design a wearable fibrous device for continuous peroxide monitoring, we propose a two-dimensional calibration curve where the ratiometric signal is a function of both analyte concentration and time. The heatmap in Figure 6. 6a demonstrates the ratiometric signal as a function of the tested concentrations of peroxide and time points. We plotted the ratiometric signal as a function of time for all concentrations (Figure 6. 6b). Interestingly, the data for all examined peroxide concentrations could be fit to single exponential association functions (Equation 1 and Figure S6. 10-S6. 11) where the offset (R_0) and time constant (τ) of the single exponential showed a narrow distribution with small standard errors of the means (Figure S6. 12 and Table S6. 2). The lack of dependence on peroxide concentration found within the fitted time constants, including the dataset of zero added peroxide, suggests the dominant physical process being modeled is that of the DNA rearrangement on the SWCNT surface. In contrast, the pre-exponential factors (A) displayed a concentration-dependent trend which could be fit to a power function (Equation 2, Figure 6. 6c and S6. 13, Table S6. 3). By incorporating Equation 2 into Equation 1, we obtained a two-input calibration function where the ratiometric signal could be expressed as a function of both peroxide concentration and time (Equation 3, Figure 6. 6d). Finally, to examine the reversibility of the platform, we exposed a sample to peroxide (200 μM) and then washed it with PBS. As shown in Figure S6. 14, we observed that the ratiometric signal increased 10 minutes after exposure to the peroxide and then completely reverts back to the original signal 50 minutes after removing it.

$$R = R_0 + A \left(1 - e^{-t/\tau}\right); R_0 = 0.383 \pm 9.28E - 4, \quad \tau = (29.305 \pm 0.615) s \quad (1)$$

$$A = (C + C_0)^p - A_0 \quad (2)$$

$$C_0 = (10.796 \pm 0.596) \mu M,$$

$$p = 0.035 \pm 2.52E - 4,$$

$$A_0 = 0.972 \pm 3.34E - 3$$

$$R = R_0 + [(C + C_0)^p - A_0] \left(1 - e^{-t/\tau}\right) \quad (3)$$

Figure 6. 6. Calibrating the optical microfibrous textiles for continuous peroxide detection. (a) Two-dimensional heat-map illustrating the ratiometric signal as a function of both concentration and time. (b) Ratiometric signal as a function of time for each peroxide concentration. The dashed lines indicate single exponential association fits. (c) Pre-exponential factors extracted from the single exponential association fits and plotted as a function of peroxide concentration. The dashed line indicates a power function fit. (d)

Contour plot demonstrating a two-dimensional calibration curve where the fitted ratiometric signal is a function of both time and concentration. Mean values were obtained by adding each peroxide concentration to three different samples ($n = 3$), and error bars represent the standard deviation.

In contrast to an average concentration reported by a single readout, the ability to spatially resolve peroxide concentrations will enable an end-user the ability to map peroxide on the surface of a wound utilizing an NIR camera with the appropriate filters. We exposed the microfibrous samples to different concentrations of peroxide and acquired hyperspectral fluorescence images from the surface of the samples under 5X magnification using the setup shown in Figure 6. 4a. By dividing the maximum intensity images of the (8,7)-SWCNT by the (9,4)-SWCNT, we created maps where each pixel represented a ratiometric signal (Figure 6. 7). At time zero, the maps for all concentrations of peroxide were dominated by blue pixels (low ratiometric signal). After 24-hour exposure to peroxide, the pixel colors among the maps diverge and are dominated by yellow color at low concentrations and red color at high concentrations. This demonstrates the potential of employing our optical fibrous platform to quantitatively image the surface of a wound in a label-free manner.

Figure 6. 7. Spatially detecting peroxide. The maps were created by acquiring NIR fluorescence hyperspectral images and dividing the max intensity image of the (8,7)-

SWCNT by the max intensity image of the (9,4) chirality SWCNT. Since the data were acquired using a 5X objective, the individual fibers cannot be observed.

To illustrate the potential of our optical fibrous platform as a smart wound dressing for *in situ* monitoring of peroxide, we attached the fibrous samples onto a commercial wound bandage (Figure 6. 8a). By attaching the samples to the complete bandage (adhesive material + adsorbent pad) or only to the adhesive material of the bandage, the fluorescence of SWCNTs was still detectable by our probe spectrometer through the bandage and with a high signal to noise ratio for each peak (Figure 6. 8b and S6. 15). The drop in the signal compared to the control is presumably due to the polymers in the bandage that absorb a portion of the excitation light and/or emitted fluorescence from the SWCNTs. Additionally, the combination of sample plus adhesive did not significantly alter the ratiometric signal even after 7 days of soaking in PBS (Figure 6. 8c). Finally, Figure 6. 8d and Movie S6. 2 demonstrate the feasibility of a real-time wireless wound screening utilizing our flexible optical fibrous platform attached onto the commercial bandage.

Figure 6. 8. (a) Integrating the optical fibrous samples into a commercial wound bandage. (b) Comparison of the fluorescence spectra of microfibers alone, through adhesive bandage material, or through both adhesive material plus an adsorbent pad (complete bandage). (c) Comparison of the ratiometric signals of the three conditions mentioned in part b, after being soaked in PBS over time. Mean values were obtained by acquiring the fluorescence spectra from three different samples ($n = 3$) per each condition. The error bars represent standard deviation. Two-sample t-tests were performed on the data (*, $p < 0.05$, **, $p < 0.01$). (d) Real-time wireless fluorescence spectra readout utilizing the flexible optical microfibers attached onto a commercial bandage.

6. 4. Conclusions

Multi-compartment smart wound dressings have attracted a substantial interest in the past few years due to their potential for enabling simultaneous wound monitoring and healing.^{51,52} A smart wound dressing usually integrates multiple layers into a single flexible biomaterial platform.⁶⁹ A therapeutic layer can be designed to enable wound healing by incorporating antibiotics, growth factors, etc., into a 3D biocompatible scaffold such as hydrogels and microfibers. Moreover, a sensing layer would enable continuous monitoring of multiple biomarkers in the wound environment. In this work, we employed a one-step coaxial electrospinning process to encapsulate ssDNA-SWCNT nanosensors into individual microfibers to fabricate wearable optical microfibrous textiles, as a sensing layer, for monitoring oxidative stress in wounds. Utilizing confocal Raman microscopy of individual fibers over time, we uncovered that the SWCNT nanosensors are preserved inside of the fibers over time and that a negligible quantity of the nanosensors are released from a 3D fibrous matrix after 21 days. As multiple nanotube chiralities in the HiPco sample respond differentially to peroxide molecules, we designed an optical wearable platform to ratiometrically detect peroxide at physiologically relevant concentrations. Utilizing this flexible optical microfibrous material, the wireless detection of peroxide was demonstrated. In addition to a single real-time readout, we demonstrated the potential of our platform for spatially resolving the peroxide concentrations on a wound surface using an InGaAs camera, without the need to use any additional marker. We finally integrated our microfibrous textiles onto a commercial wound bandage and indicated the compatibility of this platform with existing wound dressings.

6. 5. Materials and Methods

ssDNA-SWCNT Nanosensor Preparation: Raw single-walled carbon nanotubes produced by the HiPco process (1 mg, Nanointegris) were added to desalted (GT)₁₅ oligonucleotide (2 mg, Integrated DNA Technologies) in a microcentrifuge tube with NaCl solution (1 mL, 0.1 M, Sigma-Aldrich). The mixture was then ultrasonicated using a 1/8" tapered microtip (Sonics Vibracell; Sonics & Materials) for 30 min at 40% amplitude, with an average power output of 8 W, in a 0 °C temperature-controlled microcentrifuge tube holder. After sonication, the dispersion was ultracentrifuged twice (Sorvall Discovery M120 SE) for 30 min at 250 000×g, and the top 80% of the supernatant was extracted. The resultant dispersion was filtered using 100 kDa Amicon centrifuge filters (Millipore) to remove free ssDNA. A UV/vis/NIR spectrophotometer (Jasco, Tokyo, Japan) was utilized to determine the concentration using the extinction coefficient of $A_{910} = 0.02554 \text{ L mg}^{-1} \text{ cm}^{-1}$.^{32, 33}

Preparation of Core and Shell Polymer Solutions: A 4 wt.% poly(ethylene oxide) (PEO, $M_v = 900,000 \text{ g mol}^{-1}$, Sigma-Aldrich) solution was prepared by dissolving PEO in DI water and stirring the solution overnight on a hotplate set to 48 °C. A concentrated ssDNA-SWCNT dispersion (~400-500 mg L⁻¹) was prepared by filtering out the as-prepared ssDNA-SWCNT dispersion using an Amicon filter (100 kDa) and resuspending it in a lower volume of NaCl solution (0.1 M). The concentrated dispersion was then diluted in the resultant PEO solution to obtain a homogenous nanotube concentration of 10 mg L⁻¹. Because of the high ssDNA-SWCNT concentration, the final concentration of the PEO solution was not significantly altered by adding ssDNA-SWCNT dispersion to it. Polycaprolactone (PCL, $M_w = 70,000 \text{ g mol}^{-1}$, Scientific Polymer Products, Inc.) was dissolved

in a mixture of chloroform and dimethylformamide (DMF) with the volume ratio of 80:20, by stirring the solution for 6 hours at room temperature, to obtain a final PCL concentration of 13 wt.%.

Fabrication of Electrospun Optical Microfibrous Textiles: A one-step co-axial electrospinning process was used to produce core-shell fibers. Figure 1b illustrates the schematic of the experimental setup. A customized core-shell needle (Rame-hart Instrument co.) with two separate inlets was built by placing a 24 Gauge needle inside of a 15 Gauge needle. The inlets of the needle were connected to two syringes filled with the polymer solutions and placed on a syringe pump capable of controlling the flow rates separately. The flow rates of the core and shell solutions were set to 0.3 and 2 mL h⁻¹, respectively. A high voltage supply was connected to the tip of the needle and the rotating collector was grounded. The working distance between the needle and collector was set to 12 cm. To fabricate bulk fibrous textiles with a thickness of ~0.7 mm, the fibers were continuously collected on the metal collector for 7 hours. To prepare samples for NIR and confocal Raman microscopy, microscope coverslips were taped to the surface of the collector and a thin layer the fibers were collected on the coverslips for 10 minutes.

Near-Infrared Fluorescence Microscopy of the Fibers: As described previously,^{41, 70} a near-infrared hyperspectral fluorescence microscope was used to acquire fluorescence images and hyperspectral cubes from a thin layer of fibers collected on a microscope coverslip. In short, a continuous 730 nm diode laser with 2.5 W output power was injected into a multimode fiber to produce an excitation source, which was reflected on the sample stage of an Olympus IX-73 inverted microscope equipped with a 20X LCPlan N, 20x/0.45 IR objective (Olympus, U.S.A.). To generate spectral image stacks (cubes), the

emission was passed through a volume Bragg grating and collected with a 2D InGaAs array detector (Photon Etc.) with a spectral resolution of 4 nm. A background subtraction was performed using a custom MATLAB code. The background-subtracted images and hyper-spectral cubes were processed and extracted using the Fiji software.

Scanning Electron Microscopy of the Fibers: The samples were sputtered with gold prior to imaging. The SEM images were taken using a Zeiss Sigma VP field emission scanning electron microscope (FE-SEM), with an InLens detector and an accelerating voltage of 3.00 kV.

Confocal-Raman Microscopy: A thin layer of fibers collected on a microscope coverslip was imaged with a WiTec Alpha300 R confocal-Raman microscope (WiTec, Germany) equipped with a Zeiss EC Epiplan-Neofluar 100 $\times$ /0.9 air objective, a 785 nm laser source set to 35 mW sample power, and collected with a UHTS 300 spectrograph (300 lines/mm grating) coupled with an Andor DR32400 CCD detector (-61°C , 1650×200 pixels). $10 \times 40 \mu\text{m}$ areas were scanned, and spectra were obtained in $0.25 \times 0.25 \mu\text{m}$ intervals with 0.4 s integration time to construct hyperspectral images of individual fibers. Global background subtraction, cosmic-ray removal, and k-means cluster analysis were performed on each scan using WiTec Project 5.2 software. G-band intensity images were constructed by integrating the spectrum of each SWCNT-containing pixel from 1575 to 1605 cm^{-1} using custom Matlab codes.

Quantifying the Amount of the Released Nanosensor Using Solution-Based Raman Spectroscopy: 1 square inch pieces of the bulk fibrous samples with thickness of $\sim 0.7 \text{ mm}$ were soaked in 3 mL of PBS (1X) over time. The PBS was collected at different time points (1 hour, 24 hours, 48 hours, 72 hours, 7 Days, 14 Days and 21 Days) and

replaced with 3 mL of fresh PBS. The collected samples were placed into glass vials and Raman spectra were obtained using an inverted WiTec Alpha300 R confocal Raman microscope (WiTec, Germany) equipped with a Zeiss Epiplan-Neofluar 10×/0.25 objective, a 785 nm laser source set to 50 mW sample power, and collected with a UHTS 300 spectrograph (300 lines/mm grating) coupled with an Andor DR32400 CCD detector (1650 x 200 pixels). Each spectrum was averaged 5 times with 5 s integration time. Background subtraction was performed on all data using Witec Project 5.2 software.

Real-Time Near-Infrared Fluorescence Spectroscopy of Bulk Microfibrous

Samples: Bulk fibrous samples with thickness of ~0.7 mm were placed into plastic Petri dishes and 3 mL of the peroxide solution diluted to different concentrations in PBS (1X) was added to the samples. The NIR fluorescence spectra was acquired from each sample at 24, 48 and 72 hours. Individual NIR fluorescence spectra from the bulk samples were obtained using a custom-built preclinical fiberoptic probe spectroscopy system described in previous studies.^{34,35} In summary, a continuous-wave 1320-mW 730-nm laser (CNI lasers) was injected into a bifurcated fiber optic reflection probe bundle. The bundle consisted of a 200-mm, 0.22 numerical aperture (NA) fiber optic cable for sample excitation located in the center of six 200-mm, 0.22 NA fibers for collection. Long-pass filters were used to block emission below 1100 nm. The light was focused into a 193-mm focal length Czerny-Turner spectrograph (Kymera 193i, Andor) with the slit width set at 410 mm. Light was dispersed by an 1501/mm grating with blaze wavelength of 1250 nm and collected with an iDus InGaAs camera (Andor). The distance between the laser probe tip and the sample was set to 2.2 cm. A custom MATLAB code was used to perform background subtraction on the acquired fluorescence spectra.

Statistical Analysis: All curve fittings, statistical measurements and analyses were performed in OriginPro 2016. The two-sample t-tests were performed under the null hypothesis.

6. 6. Acknowledgments

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Chapter 7. Concluding Remarks and Future Directions

As mentioned in the previous chapters, the ssDNA- and polymer-wrapped SWCNTs have shown great potential for innovative molecular diagnostics applications, namely optical biosensing and bioimaging. Although they have already been utilized to design a variety of solution phase, injectable or implantable probes and devices, there is an urgent need to understand various aspects of these new nanomaterials from a physicochemical perspective. This dissertation takes a comprehensive down to the roots approach to investigate the fundamental physical, optical and material characteristics of SWCNTs non-covalently functionalized with ssDNA. Following this multifaceted characterization, we take our fundamental understanding to the next level to design the first generation of optical textiles incorporating SWCNTs for wearable biomarker monitoring.

As the initial step for the ssDNA-SWCNT nanoprobe characterization, we developed a high throughput experimental framework to quantitatively explore the complexation affinity (stability) between the ssDNA and SWCNTs as a function of the ssDNA sequence length and nanotube chirality. The complexation affinity is a crucial parameter in biosensor and bioimaging agent design, as it determines the extent of the ssDNA conformational adjustment upon biomolecule interaction with the hybrid. This is particularly important when optimization of the dynamic range, response time, sensitivity, and selectivity of a biosensor is intended. Moreover, as the ssDNA displacement from the SWCNT surface suppresses their biocompatibility, quantifying the complexation affinity is critical to select a highly stable and biocompatible hybrid for bioimaging applications. Interestingly, for the combined nanotube population, the complexation affinity strongly

correlated with the sequence length, whereas there was no systematic correlation between the complexation affinity and chirality. Utilizing our high throughput platform, we were also able to extract a complete set of fluorescence parameters that determine the ssDNA surface coverage on the SWCNTs, and we observed that these parameters do not statistically correlate with the hybrid stability; thus, the stability does not result from an increase in ssDNA coverage on the nanotube surface.

Following this comprehensive investigation on the hybrid stability, we hypothesized that intracellular processing and the fate of the ssDNA-nanotube hybrids can be manipulated by differential stabilities of the ssDNA on the SWCNT surface. To develop a fundamental understanding of the interactions between SWCNTs and live cells, we utilized a multitude of spectroscopy and imaging platforms. Our hyperspectral microscopy images demonstrated that a short ssDNA sequence ((GT)₆) experiences a significantly higher degree of intracellular optical instability compared to a long sequence ((GT)₃₀). Moreover, our intracellular hybrid stability assay indicated that the short sequence is displaced from the SWCNT surface in less than 6 hours due to competitive biomolecule absorption, whereas the long sequence remains stable for at least up to 24 hours. Interestingly, these results corroborate our previous finding about the strong correlation between the ssDNA sequence length and hybrid stability, demonstrating that our high throughput assay for complexation affinity quantification is a robust reliable platform for approximating the nanoprobe stability prior to performing *in vitro* and *in vivo* experiments. Additionally, these results suggest that the ssDNA displacement from the SWCNT surface leads to a destabilized structure within lysosomes, increasing the probability of complete ssDNA displacement or degradation from the SWCNT. Utilizing confocal Raman

microscopy, we demonstrated that the cell is able to recognize the ssDNA-displaced SWCNT as a foreign pathogenic substance and subsequently secretes its lysosomal contents. These findings accentuate that biocompatibility is a critical prerequisite in designing nanotube-based probes for long-term monitoring.

As biocompatibility is a multifaceted property, it has been demonstrated to be contingent on optimizing various characteristics of nanoparticles; thus, in addition to the surface coating and hybrid stability, the nanoparticle size is another critical parameter that determines their cellular uptake rates and intracellular fate and processing. In the case of functionalized SWCNTs, accurate size (length) quantification possesses additional advantages as their length directly correlates to their fluorescence intensity, and it also determines the number of the targeted analytes interacting with SWCNT surface in biosensing. We utilized AFM to investigate the length distributions of SWCNTs wrapped in the same short or long ssDNA sequences as the previous study ((GT)₆ or (GT)₃₀). Acquiring high-resolution AFM images and quantifying the ssDNA coverage density on individual SWCNTs, we discovered an artifact in AFM-based size measurements where differing ssDNA sequences led to a differential binding process to the AFM substrate due to their distinct coverage densities. These results uncover the significance of interfacial phenomena in nanoparticle characterization, particularly in microscopy techniques. Discerning these artifacts is crucial and can lead to developing more reliable methods and protocols for nanomaterial characterization.

Further, we were able to illustrate the ssDNA conformation around the SWCNTs for the two ssDNA sequences, based on the peak-to-peak distances acquired from the high-resolution AFM images and the Pythagorean theorem. Our results reveal that the (GT)₆

strand is not long enough to have a complete turn around the SWCNTs, whereas (GT)₃₀ makes two complete turns and one partial turn around them. These results interestingly verify our findings in the two previous studies where a short ssDNA sequence results in a significantly lower hybrid stability compared to a long sequence, both in the solution phase and in cells.

In addition to their length, we interrogated another novel physical characteristic of the ssDNA-SWCNTs, their thermal decomposition behavior, because of their demonstrated significant potential as strength-reinforcing and flame-retardant fillers in polymer nanocomposites. Utilizing thermogravimetric analysis, we discovered that hybridization with ssDNA results in the elevation of the thermal decomposition temperature of purified SWCNTs in excess of 200 °C. We also revealed that non-covalent functionalization with ssDNA significantly enhances the thermal decomposition temperatures of other carbon nanomaterials as well. Moreover, we were able to manipulate the thermal decomposition behavior of the SWCNTs by modulating the ssDNA sequence and showed that higher cytosine content results in a higher degree of thermal resistance. Enhancing the thermal stability of the carbon nanomaterials will provide various new avenues for their applications in high-temperature sensing and electronics.

Developing point-of-care diagnostics technologies based on the functionalized SWCNTs requires incorporating these nanoprobe into bulk or thin-film biomaterial platforms without altering their nanoscale optical properties. This particularly requires avoiding any additional agents and hydrophobic solvents in the biomaterial fabrication process. The resultant optical biomaterials can be utilized as wearable and/or implantable devices, enabling wireless real-time monitoring of biochemical information. Moreover,

chirality-dependent fluorescence of the SWCNTs can enable incorporating multiple chiralities, each engineered to detect a particular biomarker, into a single biosensing device and/or fabricating ratiometric biosensing devices.

Although hydrogels incorporating the polymer-wrapped SWCNTs have been successfully fabricated for implantable biosensing, they usually lack the optimized mechanical properties for versatile handling and long-term usage. In this dissertation, we designed and fabricated the first generation of the wearable SWCNT-based optical microfibrous textiles featuring a core-shell morphology in which the water-soluble peroxide-sensing SWCNTs were encapsulated inside of an FDA-approved polymer shell that is soluble in an organic solvent. The fabricated microfibers showed no apparent loss of structural integrity, nanotube aggregation, or release after being soaked in PBS for the long-term. We finally calibrated our optical textiles to design smart wound dressings for continuous wireless monitoring of wound peroxide concentrations as a measure for chronicity and infection.

All in all, the experimental framework presented in this dissertation has provided a toolbox of robust spectroscopic and microscopic assays for the fundamental characterization of SWCNT nanoprobe. The future of the SWCNT-based diagnostics technologies undoubtedly relies upon utilizing this toolbox to select the proper polymer-chirality pair from a pool of candidates for a particular application. Moreover, by introducing our optical textiles, we have started a new paradigm in wearable biosensing that has the potential to resolve some of the significant drawbacks in the current technologies, notably multiplexed protein and hormone detection in wounds and sweat. Through continuous and non-invasive monitoring of biochemical information, our optical

textile technology can potentially facilitate personalized medical approaches by enabling a detailed understanding of an individual's physiology.

Based on this dissertation, I propose several future directions for my research:

- 1- Characterizing the intracellular and intercellular uptake and retention of the functionalized SWCNT-based nanoprobe in 3D tissue models such as multicellular tumor spheroids and organoids.
- 2- Developing universal conjugation chemistry processes for robust preparation of a wide range of targeted nanotube-based biosensors and bioimaging agents by non-destructively conjugating antibodies, nanobodies, and peptides to the SWCNTs.
- 3- Scaling up the SWCNT single chirality purification processes and developing and optimizing multiplexed biosensors and multicolor imaging agents.
- 4- Designing and fabricating multiplexed nanotube-based wearable biosensors for protein and hormone monitoring in wounds, sweat, and interstitial fluid.
- 5- Developing portable and wearable NIR photodetectors to facilitate the usage of wearable nanotube-based optical biosensors.

Appendix 1

Supplementary Information for Chapter 2:

DNA-Carbon Nanotube Complexation Affinity and Photoluminescence Modulation are Independent

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Figure S2. 1. Absorption spectra (normalized at 910 nm valley) for the DNA-SWCNT suspensions of (GT) n , $n = 3, 6, 9, 12, 15$ and 30 .

Figure S2.2. Excitation and emission spectra for the (GT)3/6/9/12/15/30 as a function of chirality, before and after the addition of SDC, for the (a) (8,3), (b) (7,5), (c) (10,2), (d) (9,4), (e) (7,6) and the (f) (8,6) chirality. Horizontal black line denotes the wavelength for the SDC-SWCNT sample.

Table S2. 1. Optical parameters following SDC addition, obtained from the PLE plots.
Values are mean with standard error of the mean, from three technical replicates.

Sample	I _{initial} (a.u.)	I _{final} (a.u.)	I _{final} / I _{initial}
8,3-(GT)30	10725±173	45490±60	4.241±0.0687
8,3-(GT)15	9913±117	41370±244	4.173±0.0551
8,3-(GT)12	11673±136	46720±158	4.002±0.0485
8,3-(GT)9	11464±46	44404±179	3.873±0.0221
8,3-(GT)6	7829±134	36402±63	4.65±0.08
8,3-(GT)3	6385±166	24877±105	3.896±0.102
7,5-(GT)30	23473±256	80460±236	3.428±0.0386
7,5-(GT)15	16255±244	69438±621	4.272±0.0747
7,5-(GT)12	14913±354	78735±371	5.28±0.128
7,5-(GT)9	14353±172	70731±413	4.928±0.0657
7,5-(GT)6	9270±213	51819±172	5.59±0.13
7,5-(GT)3	11970±117	30885±151	2.58±0.0282
10,2-(GT)30	14813±291	66137±255	4.465±0.0895
10,2-(GT)15	12337±60	57689±185	4.676±0.0271
10,2-(GT)12	22361±103	69367±167	3.102±0.0161
10,2-(GT)9	9406±45	59904±313	6.369±0.0453
10,2-(GT)6	9498±290	52962±116	5.576±0.17
10,2-(GT)3	7652±379	33638±118	4.396±0.218
9,4-(GT)30	19978±396	110547±682	5.534±0.115
9,4-(GT)15	10863±82	93953±474	8.649±0.0787
9,4-(GT)12	24801±68	105293±519	4.246±0.024
9,4-(GT)9	9252±165	95360±510	10.31±0.192
9,4-(GT)6	6173±319	81061±213	13.13±0.68
9,4-(GT)3	8029±779	56342±152	7.017±0.681
7,6-(GT)30	28432±606	116787±682	4.108±0.0908
7,6-(GT)15	22725±479	102503±990	4.511±0.105
7,6-(GT)12	26223±735	116243±786	4.433±0.128
7,6-(GT)9	14510±291	104727±718	7.217±0.153
7,6-(GT)6	8678±376	77151±374	8.89±0.387
7,6-(GT)3	14726±416	48213±143	3.274±0.0931
8,6-(GT)30	13585±549	82232±960	6.053±0.255
8,6-(GT)15	5891±146	68255±1016	11.59±0.335
8,6-(GT)12	3998±84	78584±1060	19.66±0.491
8,6-(GT)9	6344±183	76589±1166	12.07±0.394
8,6-(GT)6	6001±571	75938±763	12.65±1.21

8,6-(GT)3	5858±763	46996±507	8.023±1.05
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Table S2. 2. Optical parameters following SDC addition, obtained from the PLE plots. Values are mean with standard error of the mean, from three technical replicates.

Sample	E22_initial (eV)	E22_final (eV)	Δ E22 (meV)	E11_initial (eV)	E11_final (eV)	Δ E11 (meV)
8,3-(GT)30	1.854±0.00036	1.859±0.00014	5.79±0.39	1.28±0.00003	1.288±0.00001	8.02±0.03
8,3-(GT)15	1.854±0.00019	1.86±0.00012	5.98±0.22	1.281±0.00001	1.288±0.00001	7.24±0.02
8,3-(GT)12	1.852±0.00006	1.86±0.00009	7.57±0.11	1.28±0.00001	1.288±0	8.22±0.01
8,3-(GT)9	1.856±0.00009	1.859±0.00007	3.29±0.11	1.279±0.00001	1.288±0.00001	8.53±0.01
8,3-(GT)6	1.852±0.00005	1.86±0.00005	7.49±0.07	1.279±0.00001	1.288±0.00001	8.51±0.01
8,3-(GT)3	1.85±0.000036	1.859±0.00012	9.35±0.38	1.28±0.00006	1.288±0.00002	8.06±0.06
7,5-(GT)30	1.908±0.00027	1.915±0.00014	6.95±0.3	1.196±0.00004	1.203±0.00001	7.21±0.04
7,5-(GT)15	1.91±0.000013	1.915±0.00012	5.66±0.17	1.195±0.00006	1.203±0.00001	8.42±0.06
7,5-(GT)12	1.909±0.00008	1.915±0.00011	5.8±0.13	1.193±0.00002	1.203±0.00001	10.1±0.03
7,5-(GT)9	1.91±0.00009	1.915±0.00006	5.05±0.1	1.193±0.00004	1.203±0	9.73±0.04
7,5-(GT)6	1.908±0.00013	1.915±0.00003	7.12±0.13	1.192±0.00005	1.203±0.00001	11.32±0.05
7,5-(GT)3	1.904±0.00031	1.915±0.00005	10.56±0.31	1.19±0.00005	1.203±0.00003	13.12±0.06
10,2-(GT)30	1.67±0.000013	1.676±0.00011	6.07±0.17	1.158±0.00003	1.169±0.00001	11.22±0.03
10,2-(GT)15	1.671±0.00012	1.676±0.00007	5.19±0.14	1.158±0	1.169±0	11.36±0
10,2-(GT)12	1.671±0.00014	1.676±0.00006	4.64±0.15	1.156±0.00002	1.169±0.00001	12.97±0.02
10,2-(GT)9	1.671±0.00014	1.676±0.00006	5.38±0.15	1.157±0.00002	1.169±0	11.83±0.02
10,2-(GT)6	1.67±0.00005	1.676±0.00007	5.42±0.09	1.155±0.00004	1.169±0	14.45±0.04
10,2-(GT)3	1.667±0.00027	1.675±0.00005	8.75±0.28	1.159±0.00001	1.169±0.00001	9.86±0.1
9,4-(GT)30	1.702±0.00014	1.71±0.00004	8.21±0.15	1.103±0.00001	1.116±0.00001	13.33±0.1
9,4-(GT)15	1.701±0.00031	1.711±0.00006	9.76±0.32	1.102±0.00007	1.116±0.00001	13.87±0.07
9,4-(GT)12	1.701±0.00021	1.71±0.00009	9.21±0.23	1.105±0.00007	1.116±0.00001	11.21±0.07
9,4-(GT)9	1.702±0.00036	1.711±0.00005	9.1±0.36	1.103±0.00001	1.116±0.00001	13.31±0.01
9,4-(GT)6	1.701±0.00005	1.71±0.00002	9.68±0.05	1.102±0.00003	1.116±0.00001	14.12±0.03
9,4-(GT)3	1.7±0.000063	1.71±0.00007	10.01±0.63	1.101±0.00001	1.116±0.00001	14.93±0.1

7,6-(GT)30	1.9±0.0002 4	1.906±0.00 02	6.01±0.31	1.091±0.0 0009	1.1±0.0000 1	9.18±0.09
7,6-(GT)15	1.901±0.00 018	1.906±0.00 021	5.62±0.27	1.091±0.0 0004	1.1±0.0000 1	9.58±0.04
7,6-(GT)12	1.899±0.00 013	1.906±0.00 019	7±0.23	1.092±0.0 0009	1.1±0.0000 1	7.62±0.09
7,6-(GT)9	1.902±0.00 007	1.906±0.00 016	3.95±0.18	1.09±0.00 002	1.1±0.0000 1	10.75±0.02
7,6-(GT)6	1.898±0.00 017	1.906±0.00 009	7.74±0.19	1.09±0.00 006	1.1±0.0000 1	9.85±0.06
7,6-(GT)3	1.896±0.00 047	1.905±0.00 012	9.35±0.48	1.09±0.00 001	1.1±0.0000 1	10.49±0.01
8,6-(GT)30	1.71±0.000 2	1.722±0.00 008	11.74±0.22	1.039±0.0 0006	1.05±0.000 02	10.76±0.07
8,6-(GT)15	1.711±0.00 038	1.723±0.00 012	12.03±0.4	1.04±0.00 006	1.05±0.000 02	10.69±0.06
8,6-(GT)12	1.706±0.00 038	1.722±0.00 014	16.5±0.41	1.042±0.0 0018	1.05±0.000 01	7.75±0.18
8,6-(GT)9	1.712±0.00 019	1.723±0.00 014	11.06±0.23	1.039±0.0 0006	1.05±0.000 01	11.39±0.06
8,6-(GT)6	1.715±0.00 008	1.722±0.00 006	7.19±0.1	1.039±0.0 0006	1.049±0.00 002	10.77±0.06
8,6-(GT)3	1.707±0.00 058	1.722±0.00 008	15.01±0.59	1.039±0.0 0016	1.049±0.00 002	10.12±0.16

Figure S2.3. Intensity and wavelength kinetics for all DNA-sequences for the (8,3), (8,5) and (10,2) chiralities.

Figure S2.4. Intensity and wavelength kinetics for all DNA-sequences for the (9,4), (7,6), and (8,6) chiralities.

Figure S2. 5. (a) Spectra of (8,6)-(GT)30 following SDC addition. (b) Lorentzian fits of the data for DNA-SWCNT and SDC-SWCNT. (c) Fits of the temporal evolution of the spectra as a linear sum of the DNA-only and SDC-only emission. (d) Temporal evolution of the DNA-only and SDC-only contribution. (e) Intensity and wavelength as a function of time following SDC addition.

Table S2. 3. Single exponential fits to intensity vs time. * indicate a better fit with a sum of two single exponentials: $y = y_0 + A e^{-x/t}$

Sample	y0	A	t (s)	R ²
8,3-(GT)30	3.956±0.352	-2.928±0.01613	17.41±0.1506	0.97
8,3-(GT)15	4.588±0.9396	-3.826±0.03942	5.562±0.08516	0.98
8,3-(GT)12	4.078±0.3964	-2.31±0.01565	28.82±0.3667	0.96
8,3-(GT)9	4.7±0.7185	-3.245±0.02688	15.49±0.2054	0.97
8,3-(GT)6	4.992±0.8304	-2.922±0.0242	17.6±0.2402	0.98
8,3-(GT)3	6.964±0.2069	-5.648±0.0899	2.016±0.04898	0.84
7,5-(GT)30	2.231±0.2037	-2.702±0.01466	14.64±0.1231	0.98
7,5-(GT)15	3.305±0.4003	-2.702±0.01466	14.64±0.1231	0.98
7,5-(GT)12	4.608±0.5281	-4.038±0.01714	36.44±0.3308	0.97
7,5-(GT)9	5.103±0.5146	-4.022±0.02228	10.72±0.09133	0.98
7,5-(GT)6	6.412±1.202	-4.739±0.04352	7.223±0.1001	0.98
7,5-(GT)3	2.73±0.1013	-2.695±0.009961	18.26±0.1213	0.98
10,2-(GT)30	3.776±0.04913	-3.755±0.003741	83.25±0.2368	0.99
10,2-(GT)15	4.873±0.1259	-4.722±0.008085	47.33±0.2364	0.99
10,2-(GT)12	3.724±0.1422	-3.646±0.004544	44.03±0.1385	0.99
10,2-(GT)9	6.767±0.1866	-6.904±0.01077	20.69±0.05909	0.99
10,2-(GT)6	7.484±0.09339	-7.735±0.007738	32.62±0.07492	0.99
10,2-(GT)3	5.884±0.3044	-2.657±0.01801	32.42±0.5482	0.95
9,4-(GT)30	6.211±0.07321	-6.187±0.005048	41.58±0.08642	0.99
9,4-(GT)15	9.891±0.1091	-10.12±0.009711	46.32±0.1062	0.99
9,4-(GT)12	4.187±0.07021	-4.321±0.004145	35.64±0.07431	0.99
9,4-(GT)9	11.95±0.4075	-11.54±0.02457	19.96±0.07669	0.99
9,4-(GT)6	16.94±0.4354	-15.05±0.06031	20.01±0.142	0.98
9,4-(GT)3	13.61±0.7373	-12±0.0563	12.49±0.09537	0.98
7,6-(GT)30	2.386±0.1729	-2.208±0.005143	58.56±0.3523	0.98
7,6-(GT)15	3.455±0.3332	-3.079±0.009755	44.93±0.3126	0.98
7,6-(GT)12	3.285±0.22	-2.858±0.01396	20.73±0.1704	0.97
7,6-(GT)9	9.144±0.7118	-9.331±0.0287	19.11±0.09773	0.98
7,6-(GT)6	12.9±3.172	-11.38±0.1419	11.14±0.2152	0.97
7,6-(GT)3	4.526±0.2106	-4.436±0.01726	10.09±0.06199	0.98
8,6-(GT)30	3.794±0.01028	-3.736±0.002691	74.38±0.1627	0.99
8,6-(GT)15	8.433±0.275	-8.246±0.0131	44.28±0.191	0.99
8,6-(GT)12	12.17±0.3908	-11.66±0.02337	67.54±0.4294	0.99
8,6-(GT)9	11±0.3563	-10.28±0.03018	29.66±0.1926	0.98
8,6-(GT)6	16.93±1.058	-16.69±0.0663	59.29±0.7494	0.97
8,6-(GT)3	10.61±0.4025	-7.346±0.06028	8.95±0.1146	0.95

Table S2. 4. Double exponential fit to a subset of intensity vs time

$$y = y_0 + A_1 e^{-x/t_1} + A_2 e^{-x/t_2}$$

Sample	y_0	A_1	t_1	A_2	t_2	R^2
			(s)		(s)	
8,3-(GT)12	4.145±0.3964	-1.866±0.01442	40.5±0.5857	-1.776±0.03635	2.186±0.084	0.98
8,3-(GT)9	4.79±0.7176	-2.477±0.05747	4.984±0.2147	-1.607±0.05306	33.82±1.272	0.99
8,3-(GT)3	7.305±0.2077	-5.875±0.07159	1.237±0.02654	-0.8869±0.02289	64.13±5.628	0.94
7,5-(GT)6	6.469±1.205	-4.011±0.07122	2.012±0.07081	-2.192±0.05858	16.35±0.4384	0.99
10,2-(GT)3	5.955±0.3044	-3.341±0.04606	0.3395±0.009556	-2.492±0.00832	38.36±0.3607	0.99
7,6-(GT)6	12.9±3.172	-7.076±0.6262	4.485±0.5274	-5.919±0.6634	19.21±1.536	0.97

Table S2. 5. Clustering via k-means on the biplot for the first two principal components

Principal Component 1	0.68526	-0.11026	0.71991	56.58551
Principal Component 2	0.64843	-0.35771	-0.672	31.09657
Principal Component 3	0.33161	0.9273	-0.17363	12.31791

Cluster 1	Cluster 2	Cluster 3	Cluster 4
(10,2)-(GT)3	(9,4)-(GT)3	(8,3)-(GT)3	(8,6)-(GT)3
(10,2)-(GT)6	(9,4)-(GT)6	(8,3)-(GT)6	(8,6)-(GT)6
(10,2)-(GT)9	(9,4)-(GT)9	(8,3)-(GT)9	(8,6)-(GT)9
(10,2)-(GT)12	(9,4)-(GT)15	(8,3)-(GT)12	(8,6)-(GT)12
(10,2)-(GT)15	(9,4)-(GT)30	(8,3)-(GT)15	(8,6)-(GT)15
(10,2)-(GT)30		(8,3)-(GT)30	(8,6)-(GT)30
(7,5)-(GT)3		(7,5)-(GT)9	
(7,5)-(GT)6		(7,5)-(GT)15	
(7,5)-(GT)12		(7,5)-(GT)30	
(7,6)-(GT)3		(7,6)-(GT)12	
(7,6)-(GT)6		(7,6)-(GT)15	
(7,6)-(GT)9		(7,6)-(GT)30	
(9,4)-(GT)12

Figure S2. 6. Scatter plots of excitation shift vs emission shift vs intensity enhancement for each chirality, color coded by DNA sequence length, with Spearman correlation and significance noted, where present.

Figure S2.7. Time constant as a function of DNA sequence length for each chirality, with Spearman correlation and significance noted, where present.

Figure S2.8. Chirality dependent (a) Initial excitation vs sequence length, (b) initial emission vs sequence length and (c) emission vs excitation for all six chiralities.

Appendix 2

Supplementary Information for Chapter 3: Biomolecular Functionalization of a Nanomaterial to Control Stability and Retention within Live Cells

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Table S3. 1. Table of physical properties for the shortest and longest sequences ((GT)₆- and (GT)₃₀- respectively) of DNA-SWCNTs used.

Property	DNA-SWCNT		Reference
	(GT) ₆	(GT) ₃₀	
Average SWCNT Length	186.6 ± 7.86 nm	180.6 ± 14.25 nm	1
Total Wrapped DNA per SWCNT Weight Ratio	0.696 ± 0.048 mg/mg	0.472 ± 0.047 mg/mg	1
SWCNT Diameter Range	0.8 – 1.2 nm		2

Figure S3. 1. Solution-based optical characterizations of DNA-SWCNTs. **(a)** Fluorescence spectra for all (GT)_n-SWCNTs diluted to 1 mg/L in PBS and normalized to area under the curve. **(b)** Absorbance spectra for all (GT)_n-SWCNTs in PBS normalized to the absorbance at 910 nm.

Figure S3. 2. Fluorescence intensities of DNA-SWCNTs in solution. Average fluorescent intensity as a function of DNA length diluted to 1 mg/L in (a) PBS and (b) cell culture media. Data is represented as mean $\pm$ s.d, with Pearson correlation coefficient (r_p), $n = 4$. ($***p < 0.001$, according to two-tailed two-sample t-test).

Figure S3. 3. Time-dependent intracellular fluorescence of DNA-SWCNTs. (a) NIR fluorescence images of macrophages after 30-minute pulse of (GT)_n-SWCNTs. Image

intensities were normalized to the initial intensity for each DNA-SWCNT. Scale bar = 30 μ m. (b) The percent of initial average fluorescent intensity for each DNA-SWCNT. Experiments were performed in triplicate and are represented as mean $\pm$ s.d. Significance is with respect to initial intensities (* p < 0.05, ** p < 0.01, according to two-tailed two-sample t-test).

Figure S3. 4. Fluorescence spectroscopy of DNA-SWCNTs. **(a)** Average fluorescence spectra of each (GT)_n-SWCNT diluted to 1 mg/L in cell culture media and **(b-f)** intracellular fluorescence spectra 0- and 24-hours after internalization for each (GT)_n-SWCNT. The intensity of each spectra was normalized to the area under the curve. All experiments were performed in triplicate.

Figure S3. 5. pH modulation of fluorescence spectra. **(a-e)** Average fluorescence spectra of each (GT)_n-SWCNT in cell culture media at pH of 7.4 or 4.5. The intensity of each spectra was normalized to the area under the curve. **(f)** Heat map representing the average change in SWCNT emission energy after decreasing the pH to 4.5 as a function of DNA sequence and chirality. (8,7)-SWCNTs were excluded due to poor Gaussian fits. All experiments were performed in triplicate.

Table S3. 2. Table of calculated peak emission energy shift for all examined (GT)_n-SWCNTs. Each peak was fit to a Gaussian function to obtain a center energy and subtracted from corresponding controls in cell culture media. n = 3.

Peak Emission Energy Shifts							
Chirality (<i>n,m</i>)	ssDNA	0h		6h		24h	
		Average (meV)	± s.e.	Average (meV)	± s.e.	Average (meV)	± s.e.
(10,2)	(GT) ₆	-1.518	0.392	3.338	1.046	5.084	0.307
	(GT) ₉	-0.278	0.053	0.595	0.510	-2.275	0.873
	(GT) ₁₂	-1.046	0.379	-2.761	0.155	-3.237	0.267
	(GT) ₁₅	-0.268	0.341	-1.500	0.611	-2.166	0.441
	(GT) ₃₀	-0.427	0.063	-1.427	0.252	-2.452	0.164
(9,4)	(GT) ₆	-2.065	0.183	5.706	0.253	0.694	0.047
	(GT) ₉	-3.130	0.313	-2.105	0.172	-5.008	0.233
	(GT) ₁₂	-6.382	0.627	-3.088	0.337	-3.239	0.164
	(GT) ₁₅	-3.354	0.058	-3.856	0.218	-4.854	0.065
	(GT) ₃₀	-3.099	0.208	-4.313	0.346	-4.768	0.084
(8,6)	(GT) ₆	-1.312	0.109	4.927	0.463	-0.019	0.230
	(GT) ₉	-1.756	0.128	-2.414	0.249	-3.538	0.052
	(GT) ₁₂	-2.528	0.347	-4.313	0.412	-5.063	0.332
	(GT) ₁₅	-1.909	0.144	-3.185	0.225	-3.885	0.156
	(GT) ₃₀	-1.437	0.287	-2.851	0.273	-3.409	0.248
(8,7)	(GT) ₆	-4.360	1.043	-3.435	1.948	-3.784	1.341
	(GT) ₉	-1.960	0.582	-6.161	2.107	-3.434	1.705
	(GT) ₁₂	-3.085	1.301	-1.402	1.464	-2.688	0.983
	(GT) ₁₅	-3.339	0.714	-3.756	0.683	-4.209	0.859
	(GT) ₃₀	-4.699	0.642	-7.720	0.920	-5.544	0.441

Figure S3. 6. Confocal Raman concentration-intensity calibration. **(a)** Raman spectrum of (GT)₆-SWCNTs diluted to 50 mg/L in PBS. **(b)** Example of average Raman spectrum from all (GT)₆-SWCNT-containing pixels in one cellular ROI. Linear fits of G-band intensity versus known **(c)** (GT)₆-SWCNT and **(d)** (GT)₃₀-SWCNT concentrations. **(e)** Example of cell ROI CCD count intensity scale converted to concentration using linear fit equation. Scale bar = 5 μ m.

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Appendix 3

Supplementary Information for Chapter 4: DNA Sequence Mediates Apparent Length Distribution in Single-Walled Carbon Nanotubes

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Figure S4. 1. Overlaid histograms for distributions of (a) Length of $(GT)_6$ -SWCNTs and $(GT)_{30}$ -SWCNTs, (b) Length of $(GT)_6$ -displaced and $(GT)_{30}$ -displaced SDC-SWCNTs, and (c) Peak to peak distances in height profiles.

Figure S4.2. Box and whisker plots for all the data obtained from four or five AFM images per sample combined together for (a) Length of (GT)₆-SWCNTs and (GT)₃₀-SWCNTs deposited onto mica, (b) Length of (GT)₆-displaced and (GT)₃₀-displaced SDC-SWCNTs

deposited onto mica, (c) Length of (GT)₆-displaced and (GT)₃₀-displaced SDC-SWCNTs spin coated onto silicon wafers, (d) N/L ratios, and (e) Peak to peak distances in height profiles. The boxes represent minimum and maximum values, and the whiskers represent 25-75 percentile data.

Figure S4. 3. (a) Absorbance spectra for (GT)₆-SWCNTs, (GT)₃₀-SWCNTs and SDC-SWCNTs (all samples diluted with a 1:10 dilution factor) to compare the dispersing ability of three wrappings with each other. (b) and (c) (6,5) nanotube peaks in normalized absorbance spectra. The blue shifts confirm that the DNA is displaced by SDC.

Figure S4. 4. DNA-SWCNTs spin-coated onto silicon wafers. AFM images of (a) (GT)₆-displaced SDC-SWCNTs and (b) (GT)₃₀-displaced SDC-SWCNTs. (c) Histograms for length distributions of (GT)₆-displaced SDC-SWCNTs ((GT)₆ to SDC) and (GT)₃₀-displaced SDC-SWCNTs ((GT)₃₀ to SDC). (d) The corresponding box and whisker plots to compare the average SWCNT lengths from each of four AFM images per sample for

(GT)₆-displaced SDC-SWCNTs and (GT)₃₀-displaced SDC-SWCNTs. (e) and (f) Box and whisker plots to compare the average SWCNT lengths of deposited samples to that of spin-coated samples. The boxes represent the minimum, maximum, and mean values from n=4 images. The whiskers represent 25-75 percentile data. A two-sample t-test was performed on the image-averaged data (***, p<0.001). Note: significant aggregations were observed in these spin-coated samples. For length distribution analysis, SWCNTs within aggregates were not counted.

Figure S4. 5. (a) Histograms for distribution of peak to peak distances for (GT)₆-SWCNTs and (GT)₃₀-SWCNTs. (b) Box and whisker plots for peak to peak distances from each of five AFM images per sample for (GT)₆-SWCNTs and (GT)₃₀-SWCNTs. The boxes represent minimum and maximum values, and the whiskers represent 25-75 percentile data.

Appendix 4

Supplementary Information for Chapter 5:

Enhancing the Thermal Stability of Carbon Nanomaterials with DNA

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Figure S5. 1. (a) Weight percent versus temperature profiles of purified SWCNTs. (b) First-derivative weight percent versus temperature profiles of purified SWCNTs. (c) Weight percent versus temperature profiles of raw HiPco SWCNTs. (d) First-derivative weight percent versus temperature profiles of raw HiPco SWCNTs. XPS data and the corresponding atomic percentages of the components of (e) raw HiPco SWCNTs at room

temperature, and (f) residual material from raw HiPco SWCNTs elevated to 700 °C for 30 minutes.

Figure S5. 2. Weight percent versus temperature profiles of (a) (GT)₆ DNA sequence, and (b) (GT)₆-SWCNTs. First-derivative weight percent versus temperature profiles of (c) (GT)₆ DNA sequence, and (d) (GT)₆-SWCNTs.

Figure S5. 3. Weight percent versus temperature profiles of (a) (GT)₉ DNA sequence, and (b) (GT)₉-SWCNTs. First-derivative weight percent versus temperature profiles of (c) (GT)₉ DNA sequence, and (d) (GT)₉-SWCNTs.

Figure S5. 4. Weight percent versus temperature profiles of (a) $(GT)_{12}$ DNA sequence, and (b) $(GT)_{12}$ -SWCNTs. First-derivative weight percent versus temperature profiles of (c) $(GT)_{12}$ DNA sequence, and (d) $(GT)_{12}$ -SWCNTs.

Figure S5.5. Weight percent versus temperature profiles of (a) (GT)₁₅ DNA sequence, and (b) (GT)₁₅-SWCNTs. First-derivative weight percent versus temperature profiles of (c)

(GT)₁₅ DNA sequence, and (d) (GT)₁₅-SWCNTs. (e) Weight percent versus temperature profiles of (GT)₁₅-SWCNTs at the heating rate of 10 °C/min. (f) First-derivative weight percent versus temperature profiles of (GT)₁₅-SWCNTs at the heating rate of 10 °C/min. (g) The temperature of the second peak (thermal decomposition temperature of SWCNTs) in the first-derivative weight percent versus temperature profiles of (GT)₁₅-SWCNTs. TGA was repeated three times (n=3) for each sample. A two-sample t-test was performed (n.s., $P > 0.05$).

Figure S5. 6. Weight percent versus temperature profiles of (a) (GT)₃₀ DNA sequence, and (b) (GT)₃₀-SWCNTs. First-derivative weight percent versus temperature profiles of (c) (GT)₃₀ DNA sequence, and (d) (GT)₃₀-SWCNTs.

Figure S5. 7. The temperature of (a) the first peak, and (b) the second peak, in the first-derivative weight versus temperature profiles of (GT)_n DNA sequences (n: 6, 9, 12, 15, 30). TGA was repeated three times (n=3) for each sample. A two-sample t-test was performed between every two DNA sequences to compare the first and second peak temperatures. There was no significant difference ($P > 0.05$) in all comparisons.

Figure S5. 8. (a) The temperature of the first peak (thermal decomposition temperature of DNA) in the first-derivative weight versus temperature profiles of (GT)_n DNA sequences (n: 6, 9, 12, 15, 30), and (GT)_n-SWCNT hybrids. The bar graph shows an increase in the thermal decomposition temperature of DNA in (GT)_n-SWCNT hybrids compared to that of (GT)_n DNA sequences. This increase is not significant ($P > 0.05$) for all of the (GT)_n DNA sequences. (b) The temperature of the second peak (thermal decomposition temperature of SWCNTs) in the first-derivative weight versus temperature profiles of purified SWCNTs and (GT)_n-SWCNT hybrids. The bar graph indicates a significant increase in thermal decomposition temperature of SWCNTs in (GT)_n-SWCNT hybrids compared to

that of purified SWCNTs. TGA was repeated three times ($n=3$) for each sample. A two-sample t-test was performed (**, $P < 0.01$, ***, $P < 0.001$).

Figure S5. 9. TEM image of (GT)₁₅ DNA sequence held at 400 °C for 30 minutes, demonstrating the char formed from DNA. A 10 mg/mL DNA solution was dried on a silicon nitride support grid. The temperature was rapidly increased to 400 °C and held constant for 30 minutes.

Figure S5. 10. Brightfield images and G-band intensity maps of (a) purified SWCNTs and (c) (GT)₁₅-SWCNTs dried on silicon wafers before and after 30-minute exposure to 700°C. Average spectra of SWCNT-containing pixels in confocal Raman area scans of (a) and (c) are shown in (b) and (d) respectively. Scale Bar = 100 μm .

Figure S5. 11. Weight percent versus temperature profiles of (a) raw MWCNTs, and (b) (GT)₁₅-MWCNTs. First-derivative weight percent versus temperature profiles of (c) raw MWCNTs, and (b) (GT)₁₅-MWCNTs.

Figure S5.12. Weight percent versus temperature profiles of (a) raw RGO, and (b) (GT)₁₅-RGO. First-derivative weight percent versus temperature profiles of (c) raw RGO, and (b) (GT)₁₅-RGO.

Figure S5.13. Weight percent versus temperature profiles of (a) raw C_{60} , and (b) $(GT)_{15}-C_{60}$. First-derivative weight percent versus temperature profiles of (c) raw C_{60} , and (b) $(GT)_{15}-C_{60}$.

Figure S5. 14. Weight percent versus time profiles of (a) purified SWCNTs, (b) (GT)₁₅-SWCNTs, (c) (CT)₁₅-SWCNTs, and (d) C₃₀-SWCNTs. The temperature was rapidly increased to 400 and 700 °C, respectively, and held constant at each temperature for 30 minutes.

Figure S5. 15. Weight percent versus time profiles of (a) (GT)₁₅ DNA sequence, and (b) C₃₀ DNA sequence. The temperature was rapidly increased to 400 °C and then 700 °C, respectively, and held constant at each temperature for 30 minutes.

Figure S5. 16. The first 10 minutes at the second step (where the temperature is rapidly increased from 400 °C to 700 °C) in the weight versus time profiles of (a) purified SWCNTs, (b) (GT)₁₅-SWCNTs, (c) (CT)₁₅-SWCNTs, and (d) C₃₀-SWCNTs. The decay processes were fitted to a single exponential function to extract the thermal decomposition rate (1/thermal decomposition time constant) of SWCNTs in DNA-SWCNT hybrids, in an instantaneous temperature increase procedure.

Table S5. 1. Parameters extracted from single exponential fittings

$$y = y_0 + Ae^{-x/t}$$

Sample	y_0	A	t	R^2
	(°C)	Minutes	Minutes	
(GT) ₁₅ -SWCNT	35.14899±	70.03354±	0.64172±	0.99
	3.440814	3.623106	0.030019	
(CT) ₁₅ -SWCNT	58.07047±	43.13829±	0.85189±	0.99
	1.352716	1.190044	0.016541	
C ₃₀ -SWCNT	46.95515±	54.79743±	1.38979±	0.99
	1.471978	2.309699	0.057981	

Appendix 5

Supplementary Information for Chapter 6:

A Wearable Optical Microfibrous Biomaterial with Encapsulated Nanosensors Enables Wireless Monitoring of Oxidative Stress

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Figure S6. 1. (a) Absorbance spectra, and (b) Fluorescence spectra of the (GT)₁₅-SWCNTs dispersion at 730 nm excitation.

Figure S6. 2. NIR broadband fluorescence images of the fibers produced with the applied voltage of 12 kV acquired at different regions.

Figure S6. 3. NIR broadband fluorescence images of the fibers produced with the applied voltage of 14 kV acquired at different regions.

Figure S6. 4. NIR broadband fluorescence images of the fibers produced with the applied voltage of 16 kV acquired at different regions.

Figure S6. 5. SEM images of the micro- and nanofibers fabricated with three different voltages of 12, 14 or 16 kV.

Figure S6. 6. Raman spectra of (a) polycaprolactone (PCL) polymer and (b) poly(ethylene oxide) (PEO) polymer.

Figure S6. 7. Confocal Raman microscopy of fibers soaked in PBS for 5 minutes and 24 hours. (a) and (d) The representative overlay of G-band intensity and brightfield images of fibers soaked in PBS for 5 minutes and 24 hours, respectively. (b) and (e) k-means clustering analyses of all spectra in each area scan, where $k = 4$ clusters (background

clusters omitted from figure). (c) and (f) The average Raman spectra obtained from each cluster of (b) and (e).

Figure S6. 8. Comparing the Raman spectra of the collected PBS samples over time with that of three standard samples with known SWCNT concentrations.

Figure S6. 9. The fluorescence spectra of the microfibrinous samples exposed to various peroxide concentrations. Each peroxide concentration was added to three different samples to confirm the reproducibility ($n = 3$).

Table S6. 1. Linear fits to the ratiometric signal versus peroxide concentration, when plotted on a log-log scale, in the range of 5 μM -5 mM for three different time points: $R = a + bC$. Values are mean with standard error of mean (SE), from three samples for each peroxide concentration.

Time Point	Intercept: $a \pm SE$	Slope: $b \pm SE$	R^2
24 hours	-0.38715 ± 0.00413	0.045 ± 0.00139	0.99241
48 hours	-0.35896 ± 0.00563	0.04996 ± 0.0017	0.99086
72 hours	-0.35609 ± 0.00508	0.05651 ± 0.00221	0.98796

Figure S6. 10. Ratiometric signal as a function of time for each peroxide concentration (0-50 μM). The dashed lines indicate single exponential association fits. Each peroxide concentration was added to three different samples ($n = 3$).

Figure S6. 11. Ratiometric signal as a function of time for each peroxide concentration (100-5000 μM). The dashed lines indicate single exponential association fits. Each peroxide concentration was added to three different samples ($n = 3$).

Figure S6. 12. Histograms indicating the distribution of (a) Offset and (b) Time constant extracted from the single exponential association fits of the ratiometric signal versus time, for all examined concentrations of peroxide.

Table S6. 2. Single exponential association fits to the ratiometric signal versus time for each peroxide concentration: $R = R_0 + A \left(1 - e^{-t/\tau}\right)$. Values are mean with standard error of mean (SE), from three samples for each peroxide concentration.

Concentration [μM]	Offset: $R_0 \pm SE$	Pre- Exponential Factor: $A \pm SE$	Time Constant: $\tau [s] \pm SE$	R^2
0	$0.3887 \pm 1.41E-04$	0.11629 ± 0.00454	33.39577 ± 0.12185	0.99365
1	0.39137 ± 0.00204	0.10605 ± 0.00498	35.87988 ± 1.9239	0.99477
5	0.38787 ± 0.00201	0.12837 ± 0.00342	32.9493 ± 1.61385	0.99214
10	0.38456 ± 0.00184	0.13064 ± 0.00398	29.39384 ± 1.29415	0.99826
20	0.38661 ± 0.00118	0.1504 ± 0.00228	30.07832 ± 1.24041	0.99739
50	0.3848 ± 0.00202	0.183 ± 0.00281	28.94404 ± 0.86832	0.99827
100	0.38052 ± 0.00158	0.20274 ± 0.00258	25.86262 ± 0.74715	0.99279
200	$0.38713 \pm 8.67E-04$	$0.2268 \pm 7.08E-04$	30.33552 ± 0.45051	0.99862
500	0.37924 ± 0.00139	0.27885 ± 0.00903	26.99243 ± 0.356	0.99615
1000	$0.3743 \pm$	$0.26379 \pm$	$23.58728 \pm$	0.98822

	0.00163	0.00418	0.76148	
2000	$0.37977 \pm 7.78\text{E-}04$	0.32639 ± 0.00155	27.53061 ± 0.21989	0.99676
5000	0.37714 ± 0.00234	$0.36604 \pm 8.48\text{E-}04$	26.72116 ± 0.70784	0.99657
	Avg = $0.383 \pm 9.28\text{E-}4$		Avg = 29.305 ± 0.615

Figure S6. 13. The pre-exponential factor extracted from the single exponential association fits and plotted as a function of peroxide concentration. The dashed lines indicate the power fits. Each peroxide concentration was added to three different samples ($n = 3$).

Table S6. 3. Power fits to pre-exponential factor versus peroxide concentration:

$$A = (C + C_0)^p - A_0$$

	C_0 [μM]	p	A_0	R^2
1	10.69924	0.03495	0.97934	0.9986
2	9.81501	0.03408	0.96994	0.99763
3	11.87454	0.03458	0.96881	0.98754
Avg	10.796 ± 0.596	$0.035 \pm 2.52\text{E-}4$	$0.972 \pm 3.34\text{E-}3$

Figure S6. 14. Reversible peroxide detection using the optical microfibrous textiles. (a) The NIR fluorescence spectra of the samples right before adding 200 μM peroxide (0 min), 10 minutes after peroxide addition and 50 minutes after removing the peroxide (the samples were washed with PBS after 10 minutes of exposure to peroxide). (b) The ratiometric signal at the three time points mentioned in part a. Mean values were obtained by repeating each condition three times ($n = 3$), and the error bars represent the standard deviation.

Figure S6. 15. Comparison of the fluorescence spectra of microfibers alone, through adhesive bandage material, or through both adhesive material plus an adsorbent pad (complete bandage). The fluorescent spectra were acquired from three different samples per each condition ($n = 3$).