**TITLE:**

**Engineering the Corona Phase Molecular Recognition of Single Walled Carbon Nanotubes**

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**SHORT ABSTRACT:**

We present a protocol for engineering the corona phase of near infrared fluorescent single walled carbon nanotubes (SWNTs) using amphiphilic polymers and DNA to develop sensors for molecular targets without known recognition elements.

**LONG ABSTRACT:**

Semiconducting single-wall carbon nanotubes (SWNTs) are a class of optically active nanomaterial that fluoresce in the near infrared, coinciding with the optical window where biological samples are most transparent. Here, we outline techniques to adsorb amphiphilic polymers and polynucleic acids onto the surface of SWNTs to engineer their corona phases and create novel molecular sensors for small molecules and proteins. These functionalized SWNT sensors are both biocompatible and stable. Polymers are adsorbed onto the nanotube surface either by direct sonication of SWNTs and polymer or by suspending SWNTs using a surfactant followed by dialysis with polymer. The fluorescence emission, stability, and response of these sensors to target analytes are confirmed using absorbance and near-infrared fluorescence spectroscopy. Furthermore, we demonstrate surface immobilization of the sensors onto glass slides to enable single-molecule fluorescence microscopy to characterize polymer adsorption and analyte binding kinetics.

**INTRODUCTION:**

Single-walled carbon nanotubes (SWNTs) are atomically thin layers of carbon atoms rolled into long, thin cylinders that exhibit unique electronic and optical properties.1 Such properties include a band-gap producing near infrared (nIR) fluorescence emission via exciton recombination that is highly sensitive to its local environment. The nIR emission of SWNTs falls within the near infrared window in which the penetration depth of light is maximal for biological tissue.2,3 Additionally, SWNTs exhibit several unique features atypical in contrast to organic fluorophores: SWNT exhibit a large Stokes shift, do not photobleach, and do not blink.4 Recently, exploiting these characteristics has led to the development of an assortment of novel molecular sensors with applications to biology.5,6 Unmodified, however, SWNTs are insoluble in water, and obtaining suspensions of individual SWNTs can be a challenge.7,8 Bundling and aggregation of SWNTs in solution can obfuscate their band-gap fluorescence,2 rendering them unsuitable for sensing applications.

Dispersing individual carbon nanotubes in aqueous solution requires modifying their surface to prevent hydrophobicity-driven aggregation.9 While covalent modification can render SWNTs water-soluble,10 as well as impart specific binding chemistry, defect sites in the SWNT lattice reduce or abate their fluorescence emission. Instead, SWNT functionalization can be accomplished by using surfactants, lipids, polymers and DNA9,11–13 that adsorb to the nanotube surface through hydrophobic and pi-pi stacking interactions. The resulting chemical environment surrounding surface-functionalized SWNTs is referred to as its corona phase. Perturbations to the corona phase can have a large impact on excitons traveling on the nanotube surface, causing modulations to SWNT fluorescence emission. It is this sensitive relationship between the corona phase and SWNT fluorescence that can be exploited to develop new molecular sensors by incorporating specific binding modalities onto the large surface area of SWNT. Perturbations to the SWNT corona phase upon binding analyte can lead to changes in the local dielectric environment, charge transfer, or introduce lattice defects, all of which can modulate the fluorescence emission of the SWNTs to serve as a signal transduction mechanism.14 This approach is used in the development of novel fluorescent sensors for the detection of many different classes of molecules including DNA15,16, glucose17 and small molecules such as ATP,18 reactive oxygen species19 and nitric oxide.20,21 However, these approaches are limited in that they rely on the existence of a known binding modality for the target analyte.

Recently, a more generic approach to designing fluorescent sensors was developed using SWNTs non-covalently functionalized with amphiphilic heteropolymers, phospholipids, and polynucleic acids. These molecules adsorb to carbon nanotube surfaces to produce highly stable suspensions of individual SWNTs22–25 with unique corona phases that can specifically bind proteins26,27 or small molecules including the neurotransmitter dopamine.28–30 Engineering the corona phase to disperse SWNTs and specifically bind target analytes is referred to as corona phase molecular recognition (CoPhMoRe).28 The small size, low toxicity, high stability and unbleaching nIR fluorescence of CoPhMoRe SWNT sensors make them excellent candidates for *in vivo* sensing for extended time-resolved measurements.6 Recent work has shown their applications in plant tissues for optical detection of reactive nitrogen and oxygen species.31 A particularly exciting application for CoPhMoRe SWNT sensors is the potential for label free detection of neurotransmitters such as dopamine *in vivo*, where other techniques, such as electrochemical sensing or immunohistochemistry, suffer from a lack of spatial resolution, temporal resolution, and specificity.

Designing and discovering CoPhMoRe SWNT sensors has so far been restrained by the size and chemical diversity of the dispersant library, limiting the likelihood of finding a sensor for a particular target. To date, researchers have only scratched the surface of available conjugated, co-block, biological and biomimetic polymers that could serve as functionally active dispersants for SWNT sensors. Here, we present different methods for both dispersing SWNTs and characterizing their fluorescence for high throughput screening and for single SWNT sensor analysis. Specifically, we outline the procedure for coating SWNTs with polynucleic acid oligomers using direct sonication as well as how to functionalize SWNT with amphiphilic polymers through surfactant exchange by dialysis. We use (GT)15-DNA and polyethylene glycol functionalized with rhodamine isothicyanate (RITC-PEG-RITC) as examples. We demonstrate the use of (GT)15-DNA SWNTs as a CoPhMoRe sensor for the detection of dopamine. Lastly, we outline procedures for performing single molecule sensor measurements, which can be used for characterization or single molecule sensing.

**PROTOCOL:**

Caution: Please consult all relevant material safety data sheets (SDS) before use. Nanomaterials may have additional hazards compared to their bulk material counterpart. Use all appropriate safety practices including engineering controls (fume hood, noise enclosure) and personal protective equipment (safety glasses, goggles, lab coat, full length pants, closed-toe shoes).

**1. Preparation of Buffer, Surfactant and Polymer Solutions**

**1.1) Preparation of 100 mM NaCl solution**

1.1.1) Dissolve 584 mg of NaCl in 80 mL of deionized water. Add deionized water to bring total volume to 100 mL.

**1.2) Preparation of 3% sodium dodecyl sulfate (SDS) solution**

1.2.1) Dissolve 3 g of SDS in 80 mL of deionized water. Add deionized water to bring total volume to 100 mL.

**1.3) Preparation of 2% sodium cholate (SC) solution**

1.3.1) Dissolve 2 g of sodium cholate hydrate in 80 mL of deionized water. Add deionized water to bring total volume to 100 mL.

**1.4) Preparation of imaging buffer (1X Tris: 20 mM Tris, 100 mM NaCl)**

1.4.1) Dissolve 22.23 g of Tris base and 58.44 g of NaCl in 500 mL of deionized water using a magnetic stir bar and plate.

1.4.2) Carefully add concentrated HCl until a pH of 8.1 is reached.

1.4.3) Add deionized water to reach a final volume of 1 L.

**1.5) Synthesis of RITC-PEG-RITC polymer**

1.5.1) Dissolve amine difunctionalized polyethylene glycol (PEG) (5 kDa or 20 kDa, 0.1 mol/L) and rhodamine isothiocyanate (RITC, 0.2 mol/L) in 1 mL of a 1:1 mixture of dichloromethane and dimethylformamide (DMF).

1.5.2) Add 0.2 mol/L of N,N-diisopropylethylamine (DIEA).

1.5.3) After three hours, precipitate with 10X volume of diethyl ether followed by vacuum filtration.

1.5.4) Redissolve in DMF and repeat ether precipitation followed by vacuum filtration.

Note: Other isothiocyanate modified molecules (e.g. fluorescein isothiocyanate, FITC) can be attached to PEG or other amine modified polymers using a similar method.

**1.6) Preparation of pegylated-DNA (PEG-DNA)**

1.6.1) Combine 100 µL of Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) solution (0.5 M, pH 7.0) with 44.9 µL of 5’-thiol-modified DNA (1 mg/10 µL in 0.1 M NaCl) and add to 4.855 mL of deionized water.

1.6.2) Stir for 1 hour.

1.6.3) Dissolve 500 mg of methoxypolyethylene glycol maleimide in 5 mL of phosphate buffered saline.

1.6.4) Combine DNA and PEG solutions (10 mL total) and stir for 24 hours.

**2. Preparation of Single Walled Carbon Nanotube (SWNTs) Suspensions**

**2.1) Wash of SWNTs to remove catalyst and impurities.**

2.1.1) Add 200-300 mg of unwashed SWNTs into a plastic centrifuge tube containing 45 mL of deionized water.

2.1.2) Vortex the solution for 2 minutes and sonicate using a bath sonicator for 5 minutes. Note that sonicator settings vary from instrument to instrument, so check that the optical density of the SWNT solution increases (i.e. turns black) to ensure that the SWNTs are being dispersed.

2.1.3) Centrifuge the solution for 20 min at 16100 × *g* and discard supernatant.

2.1.4) Add approximately 45 mL of fresh deionized water.

2.1.5) Repeat steps 2.1.2-2.1.4 up to 8 times.

2.1.6) Remove as much water as possible being careful not to disturb pelleted SWNTs and allow SWNT pellet to air dry.

**2.2) Nucleic acid suspensions of SWNTs**

2.2.1) Dissolve nucleic acids (NA) in 0.1 M NaCl to a concentration of 100 mg/mL.

2.2.2) Remove static electricity from disposable spatula, microcentrifuge tubes and SWNT stock using an anti-static gun. In a fume hood, add 20 µL of the NA solution to 980 µL of 0.1 M NaCl followed by the addition of 1 mg SWNTs.

2.2.3) Using an ultrasonicator with 3 mm diameter tip, sonicate the solution for 10 minutes at 40% amplitude in an ice bath.

2.2.4) Centrifuge the DNA-SWNT solution TWICE for 90 minutes at 16100 x *g*. If using PEG-DNA, purify out excess and unreacted DNA and PEG using a 100 kDa spin-filter. Add enough PBS to fill the spin-filter and spin at 9300 × *g* for 1.5 min, Repeat this wash step 3 times.

2.2.5) Collect and keep the supernatant, being careful not to disturb the pellet containing CNT bundles and aggregates. Discard the pellet in accordance with institutional hazardous waste procedures appropriate for nanomaterials.

2.2.6) Measure the solution absorbance at 632 nm using a UV/Vis spectrophotometer to determine approximate concentration of suspended SWNTs using ε=0.036 L/cm\*mg in accordance with Lambert-Beer’s law and the appropriate dilution factor.

**2.3) Amphiphilic polymer suspensions of SWNTs**

2.3.1) Remove static electricity from disposable spatula, micro centrifuge tubes, and SWNT stock. In a fume hood, add 5 mg SWNTs to 5 mL of 2% SC solution (alternatively, SDS solution can be used).

2.3.2) Using an ultrasonicator with 6 mm diameter tip, sonicate the solution for 1 hour at 40% amplitude in an ice bath.

2.3.3) Centrifuge sample at 150,000 x *g* for 4 hours using an ultracentrifuge and carefully collect supernatant.

2.3.4) Dissolve 1 wt % of amphiphilic polymer (e.g. RITC-PEG-RITC) in SC-SWNT solution.

2.3.5) Dialyze the polymer-SC-SWNT solution using a 3.5 kDa dialysis membrane against 1 L of deionized water or buffer for 5 days. Change out the water or buffer after hour 2 and hour 4. A larger dialysis membrane can be used, so long as it allows removal of the surfactant of choice, but retention of both the SWNT and amphiphilic polymer.

2.3.6) Measure the solution absorbance at 632 nm using a UV/Vis spectrophotometer to determine approximate concentration of suspended SWNTs using ε=0.036 L/cm\*mg in accordance with Lambert-Beer’s law and the appropriate dilution factor.

**3. Preparation of Surface Immobilized CoPhMoRe SWNTs**

**3.1) Preparation of BSA-biotin and Neutravidin stock solutions**

3.1.1) Dissolve 10 mg lyophilized BSA-biotin in 1 mL deionized water to make a 10 mg/mL stock solution and store at 4° C.

3.1.2) Dissolve 10 mg NeutrAvidin protein (NAV, deglycosylated avidin protein) in 2 mL of deionized water to make a 5 mg/mL stock solution. Store aliquots at -20° C. Thawed aliquots can be kept for several days at 4° C.

**3.2) Prepare BSA-Biotin coated microscope slides**

3.2.1) Clean a microscope slide and 0.17 mm cover glass (or as appropriate for the microscope objective) with deionized water, followed by methanol, acetone and a final rinse of deionized water.

3.2.2) Create channels by placing several pieces of double-sided tape approximately 5mm apart on the clean microscope slide. Seal the channels by taking a long glass coverslip and pressing it onto the top of the double sided tape. Be sure it is centered on the microscope slide so the edges of the cover slip and slide are not flush.

3.2.3) Add 100 µL of BSA-Biotin stock solution to 900 µL of 1X Tris buffer to a final concentration of 1 mg/mL.

3.2.4) Flow 50 µL of the BSA-biotin solution into the channel by pipetting the solution into one end and wicking away solution at the other using a tissue. Incubate for 5 minutes followed by 3-5 flushes with 50 µL of 0.1 M NaCl.

3.2.5) Dilute 40 µL of 5 mg/mL NAV stock in 960 µL 1X Tris buffer to a final concentration of 0.2 mg/mL.

3.2.6) Flow 50 µL of the NAV solution into the channel by pipetting the solution into one end and wicking away solution at the other using a tissue. Incubate for 2-5 minutes followed by 3-5 flushes with 50 µL of 0.1 M NaCl.

3.2.7) Dilute stock solutions of suspended SWNTs in imaging buffer to a concentration of 1 mg/mL and flow 50 µL of the solution into the channel and incubate for 5 minutes.

3.2.8) Gently rinse away excess SWNTs using 50 µL of imaging buffer.

**3.3) Preparation of APTES silanized microscope slides**

Note: APTES silanized slides allow a way to immobilize negatively charged DNA-wrapped SWNTs to the surface of a glass substrate.

3.3.1) Prepare a 10% solution of (3-Aminopropyl)triethoxysilane (APTES) in ethanol.

3.3.2) Using channels made using the steps outlined in 3.2.2, flow in 100 µL of 1X Tris buffer.

3.3.3) Flush the channel with APTES solution and incubate for 5 min. Wash with 1X Tris buffer.

3.3.4) Dilute stock solutions of suspended DNA-SWNTs in imaging buffer to a concentration of 1 mg/mL and flow 50 µL of the solution into the channel and incubate for 5 minutes.

3.3.5) Gently rinse away excess SWNTs using 50 µL of imaging buffer.

**4. Fluorescence Spectroscopy and Microscopy of SWNTs**

**4.1) nIR Fluorescence Microscopy**

4.1.1) Perform imaging of surface immobilized SWNTs using laser excitation and an inverted microscope outfitted with an InGaAs sensor array for imaging.

4.1.2) Direct the laser beam to enter the back illumination port of an inverted microscope using mirrors on adjustable kinematic mounts and a pair of post-mounted irises set to the port height. Ensure the beam is level and straight by confirming it passes through both irises when placed between subsequent mirrors and before it enters the illumination port. If necessary, adjust the beam height using a periscope assembly. Remove any short-pass heat filters on the illumination port that would attenuate the beam.

4.1.3) Insert an appropriate filter cube into the microscope to reflect the excitation light into the objective and collect emission light. This typically consists of a dichroic long pass filter with a cutoff above the excitation wavelength, (e.g. 750 LP) and an emission long pass filter (e.g. 850 LP) to further minimize scattered excitation light from hitting the sensor. Additionally, the emission filter can be chosen to be selective for the emission of SWNTs of a particular chirality.

4.1.4) Perform fine adjustments to the beam alignment by inserting an appropriate alignment cube replacing the objective with two offset, frosted discs containing 1 mm pinholes. Align the beam to the center of both the lower and upper alignment discs.

4.1.5) Attach a 2D InGaAs sensor array to the side imaging port of the microscope using an appropriate adapter and a 0.5X lens if necessary to accommodate the sensor size.

4.1.6) Using a 100X oil immersion objective (1.4 NA), apply fluorescence-free immersion oil and place the immobilized SWNT sample onto the microscope stage.

4.1.7) Raise the objective until the oil contacts the bottom of the cover glass (#1.5, 170 µm thickness). Make adjustments to the objective collar if necessary for imaging conditions, e.g. temperature, glass thickness etc.

4.1.8) Slowly raise the objective with the excitation source on and monitor the fluorescence signal of the InGaAs camera. Fluorescence intensity should gradually increase as the focal plane approaches the surface and the immobilized sensors come into focus.

**4.2) nIR Fluorescence Spectroscopy**

Note: Fluorescence spectroscopy can be performed using the same microscope setup but by directing the collected light out of the microscope body and into a spectrometer and InGaAs linear array detector.

4.2.1) Using kinematic mounts and mirrors rated for nIR, direct the light path towards the entrance slit of the spectrometer. Focus the light down to a point onto the entrance slit using a focusing lens (e.g. plano-convex, f=150mm). This alignment is accomplished by attenuating the laser power to <1mW and replacing the microscope objective with a 1” mirror and using a 50/50 beam-splitting filter cube. The excitation light will then leave the microscope body through the exit port and can be used to adjust the mirrors and lens to focus the beam onto the entrance slit.

4.2.2) After replacing the microscope objective and long pass filter, place a wellplate onto the stage and record the spectra of an in-focus sample using the spectrometer and InGaAs array.

**4.3) Measuring the reversible response of (GT)15 DNA-SWNTs to dopamine**

4.3.1) Mount sensor coated channels onto the microscope stage and bring into focus using a 100X oil objective and InGaAs camera. Add 50 µL of 100 µM dopamine solution in phosphate buffered saline (PBS) to the flow channel and record the change in fluorescence intensity.

4.3.2) Wash out the dopamine solution using phosphate buffered saline and observe the change in fluorescence of the individual SWNT sensors.

**4.4) Well-plate screening for analyte response of CoPhMoRe SWNTs**

Note: Screening of different analytes can be performed using a transparent well plate controlled using a motorized stage. An ideal well plate is transparent to visible and IR and has black-sidewalls to minimize crosstalk between the wells.

4.4.1) Pipette equal volumes of suspended SWNT sensor (e.g. 5 mg/L concentration) into each well, enough to cover the bottom of the well uniformly, typically >100 µL for a 96-well plate and >30 µL for a 384-well plate.

4.4.2) Pipette analytes (e.g. 2 µL, 100 µM final volume) from screening library into the wellplates. Prepare each analyte in triplicate to account for potential well-to-well variation, fluctuations of excitation intensity, or temperature.

4.4.3) Raise the objective (e.g. 20X achroplan, 0.45 NA) while monitoring the emission spectra using the spectrometer and InGaAs array. Optimal position of the objective will put the focal plane approximately in the middle of the sample volume in the well, which should correspond to a maximum in measured intensity.

4.4.4) For each sample well, record a 1-10 s exposure to collect a spectrum.

4.4.5) Compare the emission spectra for each well to a control containing just SWNTs without additional analyte to quantify the fluorescence response.

**REPRESENTATIVE RESULTS:**

SWNTs were suspended in aqueous solution using both surfactants and amphiphilic polymers by direct sonication and by dialysis exchange. Figure 1 shows SWNTs, grown using the iron carbonyl catalyzed method, suspended via direct sonication using SDS and C26-DNA. The optical density of a SWNTs with SDS (or polymer) increases dramatically after sonication and decreases upon removal of aggregates and contaminants through purification by centrifugation (Figure 1). Measurements of absorbance at 632 nm quantified the concentration of suspended SWNT.28

The photophysical properties of the SWNT suspensions are characterized using absorbance and fluorescence spectroscopy. Figure 2 shows the absorbance and fluorescence emission spectra of SWNTs suspended using C26-DNA and (GT)15-DNA respectively. The absorbance spectra are a superposition of the individual absorbance peaks for each distinct chirality of nanotube present in the sample. Similarly, each chirality exhibits its unique fluorescence emission peak. Differences in relative emission peak intensity are a result of differences in the population distribution of chiralities as well as differences in excitation efficiency using the 721 nm laser.

The fluorescence response of (GT)15DNA-SWNTs (where *I0* is the initial SWNT fluorescence intensity and *I* is the SWNT intensity after dopamine addition) in the presence of different concentrations of dopamine is measured by monitoring the fluorescence of a sample using a spectrophotometer and InGaAs linear array (Figure 3). The fluorescence response is a function of the emission peak (Figure 3a-d), indicating that the response may be chirality specific. The fluorescence of the 1044 nm and 1078 nm peaks increase in intensity 2-fold as dopamine concentration approaches 2 µM. Figure 3e shows the intensity of the entire emission spectra of PEG-(GT)15 DNA-SWNT increase in response to the addition of dopamine.

Individual SWNTs coated using an alternative DNA sequence, C26-DNA (prepared using the same methods at (GT)15), tethered to the surface of a microscope slide are measured under laser illumination using an InGaAs camera and 100X oil immersion objective (Figure 4). Monitoring single emitters tethered to a surface can be used to verify the reversibility of sensor response by washing target molecules away using buffer solution. Total internal reflection fluorescence (TIRF) microscopy can also be used to image dye-conjugated DNA adsorbed on the SWNTs to quantify the number of DNA molecules attached to each tube through photobleaching experiments. Figure 4 shows 3 distinct bleaching events of Cy3-labeled DNA inferred from the quantized steps of the fluorescence intensity trace of a single emitter. These results indicate that three DNA molecules are attached to the SWNT.

**Figure 1: Polymer and Surfactant Suspended SWNTs.**

(a) Photograph of RITC-PEG20-RITC SWNTs suspended using 2% SC at various points of preparation. Left: SWNTs added directly to SC solution prior to sonication. Center: After 10 min of bath sonication followed by 10 min probe tip sonication at 90% amplitude followed by centrifugation. The optical density of the solution increases as SWNTs bundles are dispersed (~100 mg/L SWNT concentration). Right: After dialysis with RITC-PEG20-RITC polymer, the final concentration of RITC-PEG-RITC suspended SWNTs is ~20 mg/L. (b) SWNT suspension yield can vary depending on the polymer used to suspend the SWNT. The optical density of the solution provides a good estimate of solution-phase SWNT concentration. Pictured are different concentrations of (GT)15-DNA suspended tubes at different concentrations. From left to right: 100 mg/L, 10 mg/L, 1 mg/L, 0 mg/L.

**Figure 2: Absorption and fluorescence emission spectra of surfactant and polymer suspended SWNTs.**

(a) Representative absorbance spectra of SWNTs suspended using (GT)15-DNA by direct sonication. The concentration of SWNT is 10 mg/L. Inset: The UV region of the absorbance spectra shows the characteristic DNA absorbance peak at 260 nm. (b) Absorbance spectra of RITC-PEG20-RITC SWNTs after exchange of SC by dialysis. Inset: Absorbance of a 10X diluted sample of RITC-PEG-RITC SWNTs shows the characteristic absorbance of rhodamine. (c) Representative nIR emission spectra of SWNTs suspended using (GT)15-DNA by direct sonication (785 nm excitation).

**Figure 3: Fluorescence detection of dopamine using (GT)15-DNA wrapped SWNTs.**

(a) Fluorescence response of (GT)15-DNA wrapped SWNTs to the addition of dopamine. Samples of sensors at a concentration of 5 mg/L were excited using a 500 mW, 721 nm CW laser. The integrated fluorescence of sensor emission from 900-1350 nm increases with added dopamine concentration 1 µM to 250 µM. (b) Fluorescence emission spectra of PEG-(GT)15-DNA wrapped SWNTs before and after addition of dopamine. The concentration of sensor is 10 mg/mL to which dopamine was added to a final concentration of 100 µM. The samples were excited using a 500 mW, 721 nm CW laser. The two highest intensity peaks approximately double in intensity after addition of dopamine.

**Figure 4: Fluorescence imaging of single surface-immobilized SWNTs.**

(a) Fluorescence emission of individual C26-DNA-SWNT (red arrows) immobilized onto a silica cover slip (#1.5) using an APTES silanization procedure and imaged using a 2D InGaAs sensor array, inverted microscope with a 100X oil immersion objective (plan apochromat, 1.4 NA), and a 500 mW, 721 nm CW laser. (b) Fluorescence bleaching experiment of C26-Cy3 DNA-SWNTs tethered to a surface using APTES. The DNA strands are 3’ terminally-labeled with Cy3 prior to tube suspension. Tracking the incremental step-wise photobleaching (red fitted trace) of individual sensors is used to determine the number of DNA molecules adsorbed onto the surface of the SWNTs. Images were acquired using an inverted microscope in TIRF mode with a 100X oil immersion objective (plan apochromat, 1.4 NA), and 561 nm laser excitation. Scale bar: 10 µm.

**DISCUSSION:**

SWNTs are readily suspended in aqueous solution via direct sonication with SDS or ssDNA, as indicated by an increase in optical density provided by the colloidal dispersion of the resulting SWNT-polymer hybrid. SDS and ssDNA disperses and solubilizes bundles of SWNTs by adsorbing onto the SWNT surface through hydrophobic or pi-pi interactions. Additionally, other polymers, such as genomic DNA, amphiphilic polymers, conjugated polymers and lipids, can be adsorbed onto the surface of SWNTs by dialysis of samples suspended using SC or SDS. Hydrophilic polymers, such as PEG, can be end-modified with hydrophobic “anchors” such as RITC or FITC to enable surface adsorption of the block copolymer. For polymers that are susceptible to shearing or degradation upon exposure to the high powers of probe-tip sonication, or for polymers with low binding affinities to SWNT, dialysis is the best method to produce a stable SWNT-polymer suspension. Following polymer encapsulation, centrifugation removes large SWNT bundles, amorphous carbon, residual metal catalyst, and other insoluble contaminants to leave a uniformly dispersed sample. Typical final concentrations of dispersed SWNTs after centrifugation range between 10-100 mg/L.

Sonication power and duration can be adjusted and optimized for the particular choice of dispersant. This is a critical step in the procedure for coating SWNTs because too little or weak sonication can result in poor dispersal, while too much or too powerful sonication can lead to poor fluorescence. Typically, lower powers are necessary to minimize damage when using polymers susceptible to shearing such as DNA. Longer sonication durations or higher intensities can lead to reduction in the length of SWNTs, where SWNTs below the ~100 nm exciton recombination length become non-fluorescent. The size of the sonicator probe tip should also match the sample volume to avoid splashing and foaming for optimal results (typically provided by manufacturer). Avoid touching the probe tip to the sides of the container and place the solution on an ice block to minimize heating of the solution. Once dispersed, solutions of SWNTs are stable at room temperature indefinitely.

Absorbance and emission spectra of solutions of dispersed generated SWNTs contain multiple peaks, indicating the presence of a mixture of dispersed SWNTs of different chiralities. Alternative methods of SWNT generation or purification methods can change the chirality distribution, leading to different peak excitation and emission fluorescence spectra. Additionally, different synthesis methods can yield samples of SWNTs with different chirality population distributions. For example: cobalt-molybdenum catalyst grown SWNTs are rich in (6,5) chirality, while high pressure with iron carbonyl catalyst)grown SWNTs are rich in (7,6) chirality, leading to differences in the absorption and photoluminescence spectra.

Certain adsorbed polymers enable the specific detection of analytes by modulating the fluorescence emission of the SWNT by changing the local environment at the tube surface. This approach offers the distinct advantage over covalent attachment of binding moieties by not permanently disrupting the SWNT lattice, which can reduce fluorescence emission intensity.6,32–34 Additionally, the CoPhMoRe approach has the potential for developing antibody-free sensors for targets where there may not already exist a known binding moiety.28 Specifically, (GT)15-DNA has been shown to selectively enhance the fluorescence emission of SWNTs in the presence of dopamine, enabling its use as a dopamine sensor. Bulk fluorescence measurements show an increase of as much as 80% in peak emission for certain SWNT chiralities. Immobilizing (GT)15-DNA SWNTs on a glass slide enables fluorescence response measurements of individual (GT)15-DNA functionalized SWNT, showing that fluorescence can increase by over 3-fold for single SWNT sensors in the presence of dopamine, without any appreciable photobleaching, under continuous laser illumination. The interaction between dopamine and the SWNT sensor is reversible, as evident by the recovery of the original fluorescent signal after washing dopamine out of the microfluidic chamber with buffer. Additionally, single-molecule measurements can be a powerful characterization technique for quantifying polymer adsorption (Figure 4) or the kinetics of binding events. Also, ratiometric sensing can be achieved by chemically isolating a singular SWNT chirality with a unique excitation-emission peak, functionalizing it with (GT)15-DNA, and isolating a second SWNT chirality to be insensitive to dopamine. Monitoring both SWNT chiralities provides a steady control fluorescence channel that can be compared to the modulating fluorescence of the (GT)15-DNA SWNTs. Combining different polymers (e.g. polyethylene glycol and (GT)15-DNA) can add additional functionality such as modifying diffusivity or cellular uptake characteristics, properties that are critical when performing *in vivo* experiments.

Currently, a limitation of the CoPhMoRe approach to sensor development include polymer library development. Because binding moieties are not known *a priori*, developing a sensor for a particular target can be time intensive and require a large number of chemically varied polymers for constructing the sensor library for screening. Additionally, stability and compatibility of sensors in *in vivo* environments can vary from sensor to sensor. However, once a candidate sensor has been identified, further modification strategies can be employed to optimize properties as necessary for *in vivo* applications.

Herein, we have demonstrated a methodology for dispersing SWNTs in aqueous solutions applicable to a wide variety of dispersing agents. This approach can be used to create libraries of dispersed SWNTs for the discovery of novel new nIR sensors for small molecules and biological markers. Of particular interest are sensors for the detection of neurotransmitters, which could enable real time, spatially precise detection of these molecules in complex biological environments.

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**DISCLOSURES:**

The authors have nothing to disclose.

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