Optical Properties of Single-Walled Carbon Nanotubes Separated in a Density Gradient: Length, Bundling, and Aromatic Stacking Effects

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Single-walled carbon nanotubes (SWNTs) are promising materials for in vitro and in vivo biological applications due to their high surface area and inherent near-infrared photoluminescence and Raman scattering properties. Here, we use density gradient centrifugation to separate SWNTs by length and degree of bundling. Following separation, we observe a peak in photoluminescence quantum yield (PL QY) and Raman scattering intensity where the SWNT length is maximized and bundling is minimized. Individualized SWNTs are found to exhibit a high PL QY and high resonance-enhanced Raman scattering intensity. Fractions containing long, individual SWNTs exhibit the highest PL QY and Raman scattering intensities compared with fractions containing single, short SWNTs or SWNT bundles. Intensity gains of approximately ~1.7- and 4-fold, respectively, are obtained compared with the starting material. Spectroscopic analysis reveals that SWNT fractions at higher displacement contain increasing proportions of SWNT bundles, which causes reduced optical transition energies and broadening of absorption features in the UV–vis-NIR spectra and reduced PL QYs and Raman scattering intensities. Finally, we adsorb small aromatic species on “bright,” individualized SWNT sidewalls and compare the resulting absorption, PL, and Raman scattering effects to that of SWNT bundles. We observe similar effects in both cases, suggesting that aromatic stacking affects the optical properties of SWNTs in an analogous way to SWNT bundles, likely due to electronic structure perturbations, charge transfer, and dielectric screening effects, resulting in reduction of the excitonic optical transition energies and exciton lifetimes.

1. Introduction

Single-walled carbon nanotubes (SWNTs) are a unique class of macromolecules, conceptualized as a single graphic sheet of sp² carbon atoms, rolled into a seamless cylinder, with diameters of ~0.5–1.6 nm and lengths from tens of nanometers up to millimeters. As a result, they have a pseudo-1D electronic structure and possess sharp Van Hove singularities in their electronic density of states, giving rise to strong resonance effects. SWNTs have been applied as near-infrared (NIR) fluorophores as well as resonance-enhanced Raman scattering labels. Recent research has focused on using SWNTs as contrast agents for in vitro and in vivo biomedical imaging via photoluminescence (PL) and Raman scattering modalities within the traditional biological transparency windows (~800 nm, NIR I and 1100–1500 nm, NIR II).

Unfortunately, although SWNTs are promising for imaging applications, as-grown SWNTs are heavily bundled and insoluble in aqueous media. The use of noncovalent surfactants preserves the graphic nature of SWNTs while imparting water solubility. Despite the use of surfactants to disperse bulk SWNT material in water, it has been documented that the presence of small SWNT bundles vastly reduces SWNT PL by nonradiative energy transfer processes. These effects make bundled SWNTs poor fluorophores, whereas the QY of individual SWNTs has been reported to be as high as 20%. Researchers have developed several density gradient centrifugation (DGC) methods in order to separate, enrich, or sort SWNTs by a variety of parameters, including length, diameter, and chirality. Crochet, Clemens, and Hertel reported that the most buoyant fractions of CoMoCat SWNTs following DGC possessed the greatest PL QY, greater than 1%, and proposed from the broadening and red shift of absorption features, that bundling of SWNTs increased in aliquots taken from higher-numbered fractions.

Less has been reported about the effect of small bundles on surfactant-dispersed SWNT absorption profiles and corresponding resonance Raman scattering properties. Previously, absorption peak red shifts and resulting changes in Raman scattering intensity have been observed by contrasting aqueous dispersions of SWNTs and flocculated or solid SWNT samples. We set out with the dual goals of optimizing the PL and Raman scattering intensities of biocompatible SWNTs for in vivo and in vitro imaging applications, as well as gaining a better understanding about the composition of SWNTs suspended in water by noncovalent surfactants. Coupled with DGC, spectroscopic analysis and atomic force microscopy facilitated an in-depth investigation into the dispersity of the as-prepared sample in terms of length and degree of bundling. Additionally, we employed small aromatic molecules to mimic the environment of bundled SWNTs in order to confirm our findings. This is the first time that a systematic investigation is carried out with separated SWNTs to correlate the photoluminescence, resonance Raman scattering, and optical absorption of individual versus bundled carbon nanotubes in suspension, and importantly, our method yields SWNTs with both “bright” photoluminescence and Raman scattering intensities, highly desired for advanced in vitro and in vivo biomedical applications.

2. Experimental Section

2.1. Materials. Raw HiPCO single-walled carbon nanotubes were purchased from Unidym and used without further purifica-
tion. Note that high-purity HiPCO SWNTs lose the majority of their photoluminescence quantum yield and thus are not of interest for imaging applications. Sodium cholate hydrate 98%, Iodixanol 60% (OptiPrep), 1-pyrenemethylamine-HCl 95%, and aminopropyl-triethoxysilane (APTES) were purchased from Sigma-Aldrich. Doxorubicin was purchased from the Stanford University Inpatient Pharmacy.

2.2. Density Gradient Centrifugation (DGC) of Cholate-Suspended SWNTs. Aqueous suspensions of SWNTs were prepared by adding 1 mg of raw HiPCO product and 40 mg of sodium cholate to 4 mL of water. The mixture was bath sonicated for 1 h, followed by 1 h of ultracentrifugation (Beckman, SW55 rotor) at 50 kRPM to remove most of the bundles and large aggregates, including high-density impurities, yielding a dark suspension of mostly single SWNTs in cholate. For separation, a layered iodixanol gradient was prepared as previously described to form a 5%/10%/15%/20%/60% iodixanol step gradient, with each step having a volume of 600 mL and containing 1 wt % sodium cholate. On top of the gradient, 400 mL of cholate-suspended SWNTs were carefully layered, followed by centrifugation at 300 000g for 1 h. Separated fractions were obtained by carefully removing 100 µL at a time. The top aliquot was labeled “fraction 1,” the second, “fraction 2,” and so on. The top 24 fractions were used for analysis.

2.3. Photoluminescence versus Excitation (PLE) Spectroscopy of SWNTs. PLE spectra were taken in a home-built NIR spectroscopy setup. The excitation source was a 150 W ozone-free xenon lamp (Oriel) that was dispersed by a monochromator (Oriel) to produce excitation lines with a 15 nm bandwidth. The excitation light was focused onto a 1 mm quartz cuvette containing the sample. Emission was collected in a transmission geometry. The excitation light was rejected using an 850 nm long-pass filter (Omega). The emitted light was directed into a spectrometer (Acton SP2300i) equipped with a liquid-nitrogen-cooled InGaAs linear array detector (Princeton OMA-V). Spectra were corrected post collection to account for the sensitivity of the detector and the power of the excitation.

2.4. Near-Infrared Photoluminescence (NIR PL) Imaging of DGC-Separated SWNTs. NIR PL images were collected using a liquid-nitrogen-cooled 320 × 256, 2D InGaAs array (Princeton 2D OMA-V) that has a sensitivity from 800 to 1700 nm. The samples were excited using a 20 W, 808 nm fiber-coupled diode laser (RPMC Lasers). The excitation power density at the imaging plane was ∼0.13 W/cm² with the total excitation power being ∼8 W. The excitation light was filtered out using an 1100 nm long-pass filter (Omega) so that the intensity of each pixel represents light in the 1100–1700 nm range. UV–vis-NIR spectra of the fractionated DGC-separated samples were taken to normalize the SWNT suspensions to have the same optical density at 700 nm. Normalization in this manner led to uniform absorption profiles for all fractions, except for variations in peak energy and sharpness, as described below.

2.5. Raman Spectroscopy of DGC-Separated SWNTs. The starting cholate-suspended SWNTs and separated, fractionated SWNTs suspended in sodium cholate and iodixanol in water were normalized as above to the same optical density at 700 nm (off resonance, away from optical transition peaks). Solution-phase samples were drawn up into glass capillary tubes and immobilized on glass slides via an adhesive. A confocal Horiba LabRam HR800 Raman spectrometer equipped with a 50× long working distance objective, Rayleigh rejection edge filter, and 300 groove/mm grating was used for all Raman scattering measurements. An 80 mW, 785 nm diode laser (spot size ∼1 µm) was focused into the center of the glass capillary tube in order to maximize the SWNT signal intensity and maintain a constant scattering volume for all measurements. Four spectra were acquired for each sample with a 2 s integration for each, in order to obtain both average intensities and standard deviations. All spectra were baseline-corrected to remove glass fluorescence (broad, ∼1400 cm⁻¹).

2.6. Atomic Force Microscopy (AFM) of DGC-Separated SWNTs. Small pieces of 300 nm SiO₂ on Si were washed with acetone, isopropanol, and finally ethanol and then blown dry with air. The chips were immersed into a 2% v/v solution of aminopropyl-triethoxysilane in ethanol for 10 min at RT under gentle agitation, then rinsed with ethanol and blown dry with air. The chips were immersed into DGC SWNT fractions for 30–120 s, then rinsed with water and dried, in order to get a near-monolayer of SWNTs on the chip surface. The chips were then calcined at 300 °C for 10 min in air. A Nanoscope III multimode AFM in tapping mode (Veeco) was used for AFM imaging, and NanoScope 5 software was used for length analysis. Obviously bundled SWNTs or those that extended beyond the viewing window were excluded from analysis, as accurate lengths for these structures cannot be obtained. One must note that bundling of SWNTs may occur during the adsorption/calcination process or as a result of APTES. Moreover, deposition rates of individualized or bundled SWNTs in fractionated samples onto the modified silicon AFM substrates may not be identical. Thus, AFM images may not represent accurate depictions of SWNT bundling in solution.

2.7. Loading of Small Aromatics onto Individualized SWNTs. Doxorubicin and 1-pyrenemethylamine were dissolved in water and serially diluted from 2 mM to 2 µM. DGC-separated, sodium cholate-suspended SWNTs in water were prepared as above, and fractions 5–9, which demonstrated the most intense PL and Raman scattering properties, were pooled. Doxorubicin and 1-pyrenemethylamine were diluted 10-fold into aliquots of the SWNT suspensions along with a water control and incubated on an orbital shaker at RT for 1 h. Raman spectra were acquired as described above.

3. Results and Discussion

3.1. DGC Separation and Photoluminescence Analysis. DGC rate (zonal) separation separates molecular species primarily by size and mass, rather than density. For a given separation medium density, the sedimentation coefficient of surfactant-wrapped SWNTs is dependent upon several factors, including chirality (SWNT diameter), length, and surfactant packing, as hypothesized in previous work. Empirically, separation of sodium cholate-suspended SWNTs through an iodixanol step-gradient at ~300 000g resulted in a continual distribution of buoyant SWNTs, as well as the accumulation of some SWNTs at the “stopping” 60% iodixanol base layer (1.32 g/mL) (Figure 1a). After normalization of the separated fractions to the same optical density, NIR PL (808 nm excitation, 1100–1700 nm emission) images were used to evaluate the relative quantum yield (QY) of each fraction (Figure 1b). The resulting DGC separation of SWNTs demonstrated nonmonotonic variation in the PL QY intensity versus centrifugal displacement. The fractions containing SWNTs at the very top of the column (fractions 3 and 4) show a very low relative QY compared with the starting material. The relative QY shows a marked increase starting with fraction 5 and peaking at a value of ~170% of the starting material at fractions 6 and 7, followed by a monotonic decrease in relative QY toward the higher-numbered fractions. This result suggests that separation can lead to SWNT fractions with a nearly 2-fold increase in relative QY.
over as-prepared suspensions, which will be useful for biomedical imaging applications. Photoluminescence measured as a function of excitation energy for fractions 4, 6, 8, 14, and 20 corroborated the observed trend compared to the starting material and revealed no obvious SWNT chirality enrichment following DGC separation (Supporting Information, Figure 1). This observation was supported by UV–vis-NIR absorption measurements.

3.2. Raman Scattering Analysis Following DGC. Interestingly, resonance Raman scattering analysis, under 785 nm laser excitation, of the DGC-separated SWNT fractions showed similar relative intensity trends as the relative PL measurements (Figure 2). Obvious trends were observed for the primary Raman modes of SWNTs, including the radial breathing modes (RBMs, 100–300 cm\(^{-1}\)) and graphitic band (G-band, \(\sim 1590\) cm\(^{-1}\)). The DGC-separated and absorbance-normalized fractions of SWNTs showed a sharp rise in Raman scattering intensity from fractions 3–6, with a peak in both the RBM and the G-band scattering intensity in fraction 6, coinciding with the peak in PL QY. This peak is followed by a gradual decrease in intensity for both modes (Figure 2a,b). Similar trends were observed for Raman scattering spectra measured at 633 nm excitation (Supporting Information, Figure 2), suggesting that causes of both enhanced QY and Raman scattering intensity are not specific to a few SWNT chiralities. At the peak in fraction 6, there is a 3.75-fold increase in RBM scattering intensity (233 cm\(^{-1}\)) and a 2.5-fold increase in G-band intensity when compared with the starting material at the same optical density. No shifts in RBM or G-mode peak energies were observed from fractions 2–24 (Supporting Information, Figure 3).

3.3. Length Analysis by Atomic Force Microscopy. The rapid increase in PL QY and Raman scattering intensities from fraction 4 to 6 is accompanied by a rapid increase in median SWNT length (Figure 3).\(^1\,\!\!^{16,24}\) SWNT length enrichment by DGC rate separation results from differences in sedimentation coefficients for short and long species.\(^23\) AFM was used to measure SWNT length distribution profiles for fractions 4, 5, 6, 8, 14, and 20 (Supporting Information, Figure 4), and the most buoyant fractions appear to be composed of SWNTs with very short lengths (mostly <100 nm). Fraction 4, which has a PL QY of 0.17 and an RBM intensity of 0.46 relative to the starting material, is composed of relatively low aspect ratio SWNTs, 77% of which are less than 100 nm long with a median of 66 nm. In striking contrast, fraction 6 contains SWNTs with lengths over 800 nm, with 66% of SWNTs greater than 100 nm in length (Figure 3). These longer SWNTs show a 1.7-fold increase in
PL QY and an RBM intensity increase of 3.75-fold compared with the starting material. Fractions 5–20 contain SWNTs with a median length $>100$ nm.

PL emission is particularly sensitive to the length of the nanotube. It has been observed that the exciton diffusion length is $\sim 100$ nm. As a result, nanotubes with lengths approaching this value have reduced QYs due to the fact that excitons will be quenched by defect sites at the open ends of the nanotube. This length effect explains the very low relative QY of the most buoyant fractions (fractions 3 and 4, length mostly below 100 nm) and the marked increase in QY seen in fractions 5–9, which have lengths mostly above 100 nm. Raman scattering intensity also increases considerably between fractions 4–6, likely as a result of length separation. Previous reports have explored SWNT length effects on the Raman scattering intensity by size-exclusion chromatography (SEC). SEC revealed that Raman scattering, as well as PL QY, increases with increasing SWNT length.

### 3.4. Spectroscopic Confirmation of SWNT Bundle Sorting

Whereas the separation of short ($<100$ nm long) SWNTs in the lowest-numbered fractions is likely the cause of the rapid intensifying of SWNT optical emission processes up to the peak in fractions 6–7, the cause of the gradual loss of both PL QY and relative Raman scattering intensity in higher fractions, in which the length distribution is similar, is due to the presence of small nanotube bundles (Figure 2b). Bundling of dispersed SWNTs is known to reduce the PL QY via nonradiative energy-transfer processes. Excitons can decay nonradiatively into a neighboring metallic nanotube, leading to quenching of the photoluminescence.

Bundling causes red shifting and absorption peak broadening of the excitonic optical transitions in SWNTs. We observed a red shift of 13 nm (25 meV) for the optical transition near 800 nm (Figures 2c and 4a) with increasing fraction number. The optical transition energies of the starting material fall within the range set by the DGC-separated fractions, indicating that bundles existed in the starting material prior to DGC and are not a result of the process. Broadening of optical transition peaks was also observed, suggesting the presence of a broad distribution in the degree of SWNT bundling. Indeed, Raman scattering analysis revealed a relative increase in the (10,2) RBM intensity, indicating an increase in SWNT bundling following DGC, in fraction 7 and above (Figure 4b). This phenomenon results from increased resonance enhancement of the radial breathing mode of the (10,2) SWNT at 785 nm excitation, caused by the red shifting of optical transitions associated with bundling. Photoluminescence versus excitation (PLE) measurements (Supporting Information, Figure 1) did reveal exciton energy transfer bands, indicating that some SWNT bundles remain in the DGC fractions with the highest PL QY. However, because the HiPCO SWNT material consists of 61% semiconducting species, statistical arguments suggest that these fluorescing bundles must be small (composed of 2–3 SWNTs) in order to contain only semiconducting species. Such small SWNT bundles may sediment with rates similar to those of individualized SWNTs, and therefore, the presence of a few remaining bundles, even in the brightest fractions following DGC separation, is consistent with the DGC separation mechanism.

The relative decrease in Raman scattering intensity in higher fractions is likely related to the red shifting of the SWNT optical
transitions, following from an increasing proportion of SWNT bundles with increasing fraction number. We hypothesize that reduced resonance caused by SWNT bundling results in the gradual decline of the Raman scattering intensity. The excitation laser used herein (1.58 eV) is in near-resonance with the second excitonic optical transitions of (9,7), (10,5), (11,3), and (12,1) chirality SWNTs, with energies of 1.563, 1.574, 1.564, and 1.552 eV, respectively. As these near-resonant SWNTs present in the sample are lower in energy than the incident photons, an additional red shift of the optical transitions reduces the resonance for all four chiralities (contrary to the (10,2) “bundle peak” that is resonantly enhanced upon bundling). Because of the combination of excitation and emission resonance conditions, the lower-energy RBM peak intensities are much more sensitive to shifts in resonance than the G-mode (as seen in Figure 2b) or other higher-energy Raman scattering modes. Thus, as the optical transition peaks red shift with increasing fraction number, reductions in both the RBMs and the G-bands are observed under a constant 785 nm excitation.

3.5. Small, Aromatic Molecule Stacking Effects on “Bright” SWNT Fractions. The bundling of SWNTs in aqueous media is driven by van der Waals forces, as well as \( \pi \)-electron interactions. We employed small polycyclic aromatic molecules to mimic the SWNT bundling effect. Interaction of these molecules, possessing poor aqueous solubility, with SWNTs has been reported in the past and agrees with theoretical predictions. Simple mixing of aromatic molecules, such as doxorubicin (Dox) and 1-pyrenemethylamine (pyrene-NH\( _2 \)), with “bright” DGC-separated, cholate-suspended SWNT fractions (Figure 5a) led to a concentration-dependent red shift and broadening of absorption peaks. The degree of red shifting and the change in full width at half-maximum of the SWNT optical transition peaks were greater for Dox than pyrene-NH\( _2 \). Consequently, the addition of pyrene-NH\( _2 \) to “bright” SWNT fractions also led to a 60% decrease in RBM intensity, whereas addition of Dox reduced the RBM intensity by 75% (Figure 5b). Moreover, the addition of 200 \( \mu \)M Dox led to a 33% reduction in NIR PL intensity, whereas the addition of 200 \( \mu \)M pyrene-NH\( _2 \) led to a reduction in NIR PL intensity of only 27%.

Adsorption of aromatic molecules, like interactions of bundled SWNTs, reduces the excitonic optical transition energies of SWNTs. A greater red shift in absorption was observed for Dox, and subsequently, a greater reduction in Raman scattering intensity was observed (Figure 5). It should be noted that the drop in PL QY was less pronounced than the drop in resonance Raman scattering in the presence of the small aromatic molecules. This is likely due to the fact that, although the presence of small aromatics can allow excitons to decay due to charge screening or charge-transfer processes, they may not quench the photoluminescence as efficiently as the presence of a metallic nanotube in a bundle. We believe that this is due to the poor spectral overlap of the small aromatic molecules with the SWNT emitter. Metallic nanotubes have a continuum of electronic states, yielding nonzero spectral overlap between donor semiconducting SWNTs and acceptor metallic SWNTs in a bundle.

The bundling of SWNTs, or physisorption of small aromatic molecules onto SWNT sidewalls, perturbs the SWNT single-particle band structure and increases dielectric screening effects, which, in turn, reduce excitonic optical transitions. This effect...
is, in part, mitigated by a decrease in exciton binding energies, but overall, the band structure effects outweigh excitonic effects. Charge transfer, caused by either interactions of SWNT sidewalls or the $\pi$-density contribution of small aromatic molecules, leads to increased Coulomb interactions, and subsequent carrier charge screening, that reduces exciton lifetimes, and leads to PL quenching and contributes to the broadening of optical transitions.

4. Conclusion

In conclusion, we have performed density gradient centrifugation (DGC) of sodium cholate-suspended SWNTs in water to separate individual nanotubes from small bundles. We reveal that short SWNTs in the topmost fractions exhibit relatively low photoluminescence QY and resonance Raman scattering. Long, individual SWNTs exhibit the highest QY and Raman scattering intensities, 2- to 4-fold higher than as-made SWNT suspensions containing both single and bundled carbon nanotubes. SWNTs found located in high-numbered fractions had higher degrees of bundling, resulting in increasingly red shifted and broadened absorption peaks. This is the first time that a systematic investigation is carried out with separated nanotubes to correlate the photoluminescence, resonance Raman scattering, and optical absorption of individualized versus small, bundled nanotubes. Importantly, our method obtains fractions of “bright” nanotubes, with the highest photoluminescence quantum yield and Raman scattering abilities for advanced in vitro and in vivo biomedical applications.

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Supporting Information Available: Photoluminescence versus excitation of selected DGC fractions and additional DGC Raman analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes