Water-Soluble DNA-Wrapped Single-Walled Carbon-Nanotube/Quantum-Dot Complexes**

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In recent years, carbon nanotubes (CNTs), especially single-walled carbon nanotubes (SWCNTs), have attracted much attention due to their unique properties and potential towards broad real-world applications. The integration of SWCNTs with other unique nanoscale luminescent materials, such as quantum dots (QDs), has enabled the manufacture of many novel nanocomposite materials with enhanced structural, mechanical, optical, and chemical properties.\[1,2\] The performance of these composite materials strongly depends upon the properties of the individual components and additives as well as the conjugation chemistry required to assemble them into composite hybrids. Therefore, a variety of new techniques\[3–9\] have been developed to modify the optical, mechanical, chemical, and electrical properties of SWCNTs to control the properties of the final composite materials.

Among the additives to SWCNT-based composites, novel nanoparticles (NPs) have been increasingly employed. Functionalized NPs can be designed to covalently bind to the functional groups expressed on the sidewalls or ends of SWCNTs.\[10–16\] Their binding efficacy and the nature of the interactions (e.g., covalent bonding, physisorption) may be monitored by optical spectroscopy techniques. Accordingly, NPs might serve as probes to measure the distribution of target functional groups on SWCNTs. Core/shell fluorescent semiconductor NPs or QDs, such as cadmium selenide/zinc sulfide (CdSe/ZnS) and cadmium telluride/zinc sulfide (CdTe/ZnS), have been used for this approach because of the following advantages. They provide unique fluorescence properties, such as broad absorption and narrow intense size-dependent emission bands, thus enabling multiplexed imaging of QD distributions in the hybrid QD–SWCNT system by fluorescence microscopy. Additionally, various techniques to functionalize QDs with many chemical groups are readily available, and the influence of functionalization on their optical characteristics has been characterized.\[17\] Other imaging techniques, such as electron microscopy and confocal Raman microscopy, also allow visualization of SWCNT and QD distributions.

QDs have been widely employed in manufacturing hybrid nanomaterials with either multi-walled carbon nanotubes (MWCNTs)\[10,14,15,18,19\] or SWCNTs.\[11–13,16,20,21\] Applications of these hybrid materials include nanophotonics, molecular electronics, and chemical and biological sensors. However, the manufacture of these materials for reliable real-world products is still challenging, since techniques to precisely control the modification and monitor the efficacy of conjugation processes have not been fully achieved, although technologies have been advancing to this end.\[20,22\] Furthermore, for enhanced design and engineering strategies, modeling of CNT structure and interactions with their nanoscale environment has also become increasingly important.\[13,14\] In particular, the study of photoinduced charge transfer between the QD and the CNT has drawn much attention as applications of these materials in electro-optic devices are becoming feasible. Since these models are centered on single CNT levels, comparison to relevant experiments requires that the conjugate materials be at single CNT levels. At the same time, the conjugation chemistry should not perturb the unique intrinsic properties of each component in the composite of QDs and CNTs. To minimize the perturbation, functionalization of CNTs by physical adsorption of functional-group-attached polyaromatic hydrocarbons to the CNT surface has been suggested.\[18–20\] However, instability of these physisorbed...
hydrocarbons often prevents further reliable conjugation with QDs functionalized with counterpart functional groups, and these hydrocarbons often modify the chemical properties of SWCNTs which makes quantitative spectroscopic analysis challenging in understanding the detailed interactions between QDs and SWCNTs. Recently, a novel approach to conjugate water-soluble QDs functionalized with carboxylic acid groups to SWCNTs wrapped with DNAs functionalized with biotin, thiol, or dithiol groups has been demonstrated.[23] In this study, functionalized QDs that target-labeled DNAs on SWCNTs revealed previously undescribed nanoscale details on the interactions between DNAs and SWCNTs, especially the location of oligonucleotides on the CNT.

Herein, we report a new approach to effectively conjugate QDs onto dispersed SWCNTs through covalent amidation, by employing single-stranded DNA oligonucleotides with guanine (G) and thymine (T) repeating units as linkers between the QDs and CNTs (Figure 1). It has been shown elsewhere that these linker DNA molecules are attached to the surface of CNTs through physisorption with minimal modification to the intrinsic properties of the tubes,[24,25] and result in excellent dispersion of SWCNTs in aqueous solutions.[26–29] These highly dispersed SWCNTs may enhance the binding efficiency between QDs and SWCNTs and allow for detailed study of their interactions in a well-dispersed state. Additionally, the well-understood spectral characteristics of DNA molecules enable a nonobscured subtraction of background signals. This allows differentiation of SWCNT and QD signals from the background signal, which enables thorough study of their interactions involving charge transfer between SWCNTs and QDs.

The final conjugation was achieved by amide bonding between carboxyl-QDs and amine-DNA molecules physisorbed onto SWCNTs. A successful amidation reaction was confirmed by the Fourier transform infrared–attenuated total reflectance (FTIR-ATR) spectra[30] (Figure 2A) by monitoring the evolution of vibrational bands of the functional groups. Before conjugation, the COOH-QD spectrum shows a weak band near 1736 cm\(^{-1}\) from carboxylic acid C=O stretching. The bands at 1643 and 1562 cm\(^{-1}\) are assigned to the C=O stretching and NH bending modes, respectively, of the amide group. This indicates that the QDs contain substantial amide motifs in the linker arm with a carboxyl group at the end. The spectrum of DNA–SWCNTs, prior to conjugation, contains two sharp bands at 3182 and 1630 cm\(^{-1}\) due to the stretching and bending modes, respectively, of the primary aromatic amines of the guanine bases. These DNA–SWCNTs also show a band at 1552 cm\(^{-1}\) due to CNH bending of the DNA. After conjugation by the amidation reaction (Figure 2A, spectrum c), vanishing of the DNA–SWCNT amine bands (3182 and 1630 cm\(^{-1}\)) and the QD carboxylic acid band (1736 cm\(^{-1}\)) is evidence of amide bond formation between DNA and QDs. Furthermore, the spectrum of the conjugate shows two broad peaks at 1546 and 1641 cm\(^{-1}\), which could be attributed to N–H bending and C=O stretching modes of the DNA–SWCNT/QD amide linkages. However, amide motifs in the QD linker arms may also contribute to these signals.

To be further confident that these peaks are mostly from amide linkages, we conducted an alternative amidation reaction using amine-QDs conjugated to carboxyl-SWCNTs, by switching counterpart functional groups on the QDs and SWCNTs. Indeed, spectral analysis on this conjugated material indicated the same signatures indicative of amide linkage between the QDs and SWCNTs (Figure 2B). In detail, the

Figure 1. Schematic diagram of the conjugation of DNA-wrapped SWCNTs and COOH-QDs. EDC = 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; sulfo-NHS = sulfo-N-hydroxysuccinimide.

Figure 2. FTIR spectra of reactants and conjugated products. A) Spectra of DNA–SWCNT/COOH-QD samples: a) COOH-QDs; b) DNA–SWCNTs; and c) DNA–SWCNT/QD conjugates. B) Spectra of COOH-SWCNT/NH\(_2\)-QD samples: a) NH\(_2\)-QDs; b) COOH-SWCNTs; and c) COOH-SWCNTs/NH\(_2\)-QDs complex. The top and bottom spectra were obtained by the FTIR-ATR technique; the middle spectra were collected by transmission FTIR using a KBr pellet.
features that support the formation of amidation linkages of amine groups on the QDs. After conjugation, the spectral \( C - O \) from NH2 on the QD. 

A broad peak at around 3400 cm\(^{-1}\) is obvious in this sample due to hydrogen-bonded OH stretching of adsorbed water. The amine-QD sample exhibits a free NH bending mode at 1656 cm\(^{-1}\) from the amine groups on the QDs. After conjugation, the spectral features that support the formation of amidation linkages between NH2-QDs and COOH-SWCNTs include three characteristic peaks associated with the \(-CONH\) group at 1646, 1534, and 1237 cm\(^{-1}\), a substantially decreased SWCNT \( C=O \) peak at 1735 cm\(^{-1}\), and the band at 1656 cm\(^{-1}\) originating from NH2 on the QD.

In addition to using spectroscopic features to provide strong evidence of amidation conjugation, imaging techniques have been used to view the dispersion and conjugation distribution of the material. The distribution of QDs on the DNA-wrapped SWCNTs is readily observed by transmission electron microscopy (TEM), as shown in Figure 3. QDs are clearly seen as dark round dots distributed preferentially along with the SWCNTs. In the areas that have multiple QDs, association (“bundles”) of SWCNTs is also observed, where neighboring SWCNTs are bridged by conjugation to the same OD with multiple carboxylic groups on the OD surface. To evaluate the size of single QDs within our TEM resolution (\( \approx 0.5 \) nm), we estimated the diameters of dark round features on the nanotubes, as shown for one area of Figure 3C. The estimated sizes of the features with a diameter less than 7 nm are displayed in the inset of Figure 3. We believe that features larger than \( \approx 7 \) nm can be attributed to aggregates of multiple QDs, within which the resolution of individual QDs is difficult by our TEM instrument. The diameter of each dark circular feature was determined by averaging three different measurements of the full width at the 1/e\(^2\) local minimum of the grayscale intensity along the lines across the dark spot. Within the uncertainty of our TEM measurement and taking into account the aspect ratio (\( \approx 1.4 \)) of the QDs in use, measured diameters of five different dark features are in the range of 3.1–4.0 nm, which is in good agreement with other TEM results reported elsewhere.\(^{[3,5]}\) To further support the evidence for amidation conjugation between QDs and DNA–SWCNTs, we also conducted control experiments on the DNA–SWCNTs and QDs by mixing them in the absence of the conjugation reagents. TEM and atomic force microscopy (AFM) images of these samples adsorbed on a TEM grid or a mica substrate exhibited dispersed QDs sequestered from DNA–SWCNTs, indicative of negligible nonspecific interactions between the materials (Figure 4). Lastly, we conducted optical analysis of these samples in an aggregated form by physisorption, and the results are discussed later.

We observed that the prepared DNA–SWCNT/QD conjugates still retain their water solubility and that dispersions are stable for several months after the initial preparation of the conjugates. Additionally, we confirmed that the SWCNTs in this conjugate solution still exhibit the characteristics of the dispersed state, although they appear to be “bundled” SWCNTs due to multiple carboxylic groups on a QD, which draws neighboring SWCNTs together. To this end, UV/Vis/near-infrared (NIR) absorption spectroscopy was used to assess the dispersion state of the SWCNTs in the conjugates in water. It is well known that well-dispersed SWCNTs show strong peaks in the UV/Vis/NIR region due to the characteristic absorption between the Van Hove singularities in the conductance and valence bands,\(^{[33]}\) whereas tightly bundled SWCNTs in contact display nearly no peak features between 200 and 1400 nm. Figure 5A shows the UV/Vis/NIR spectrum of DNA–SWCNT/QD conjugates dispersed in water, together with the spectra of their reactants, DNA–SWCNTs and NH2-QDs, before conjugation. A comparison of these three spectra suggests that the conjugates (top spectrum) continue to display the characteristic peaks of dispersed DNA–SWCNTs (middle spectrum), and remain nearly unaffected by the attachment of the QDs (bottom spectrum), although some peak broadening is observed probably due to association of SWCNTs by bridging QDs. The high solubility in water is not surprising since small “bundles” of SWCNTs with QDs are expected to be well-dispersed in aqueous solution due to the large number of hydrophilic groups on the surface, such as N–H, PO4, and C=O, in the DNA nucleotides, which can readily form hydrogen bonds with water. Additionally, the attachment of QDs does not necessarily reduce the water solubility of the DNA-wrapped SWCNT conjugates, due to sufficient remaining surface COOH groups on QDs even after conjugation. Knowing that the intrinsic physical properties of SWCNTs and QDs are well retained through the conjugation process, we became confident that any changes in the characteristics of QDs and SWCNTs in the conjugate

Figure 3. TEM images of the DNA–SWCNT/QD complex. QDs are the dark spots on the SWCNT lines. More QDs are seen in the areas where SWCNTs appear to be bundled, which indicates that single QDs have multiple linker arms to bridge multiple SWCNTs. Inset: estimated diameters (nm) of single QDs shown within the boxed area in (C). Scale bars: 40 nm (A, C) and 30 nm (B).
samples are attributable to the interaction between SWCNTs and QDs.

In recent studies, the possibility of charge transfer between SWCNTs and QDs in conjugated materials has been a controversial issue, mainly due to the ambiguity of the structure of samples in use for measurements. Here, as our initial spectroscopy data provide details of the structural information, a careful analysis of the highly resolved UV/Vis/NIR absorption and fluorescence spectra of the conjugates provides evidence that supports charge transfer between QDs and SWCNTs. It is known that the density of states (DOS) of SWCNTs divides into a series of the so-called Van Hove singularities because of the radial confinement of the wave function (see Figure 5B). Each distinct chirality of the SWCNT poses its own unique series of DOS, although these can be modified by the environment, impurities, or attachments. The electronic transition between these singularities provides the eminent features in optical spectra.

The major advantage of our well-dispersed conjugates over those in previous work is that our samples can be optically excited to give rise to sharp peaks of dispersed SWCNTs in the UV/Vis/NIR spectrum, which enabled us to resolve the change in the energy gap for each distinct species of CNTs induced by QD conjugation. In Figure 5A, the UV/Vis/NIR peaks of the DNA–SWCNT/QD conjugates exhibit a red shift of approximately 1 to 10 nm (except at 508 nm) relative to their original positions for SWCNTs prior to conjugation with QDs. These red shifts can be explained by charge transfer from the donor QDs to the acceptor SWCNTs, which alters the DOS of SWCNTs to lower the band gap. It appears that the peaks at 598 and 1142 nm of the conjugate overlap with those of QDs near the same wavelengths (599, 1153 nm), thus implying some contribution from QDs (Figure 5A, QDs-COOH curve). However, the final retained concentration of QDs in the final conjugate sample is orders of magnitude less than that of the QD-only solution from which the spectrum in Figure 5A was obtained. Therefore, the contribution from unconjugated QDs to these peaks is negligible. Furthermore, the extent of change for the SWCNT interband gap due to the conjugation is estimated by averaging the wavelength variation (except for 508 nm), that is, (7.1 ± 0.6) meV for the first interband transition (Sv1→c1) and (7.1 ± 3.4) meV for the second interband transition (Sv2→c2) of the semiconducting SWCNTs, and (8.5 ± 4.3) meV for the first interband transition (Mv1→c1) of the metallic SWCNTs. The slightly higher interband change for the metallic SWCNTs than that for their semiconducting counterparts might be attributable to the larger functional coating.

To investigate further details of the charge-transfer mechanism, fluorescence microscopy and spectroscopy studies on QDs in the DNA–SWCNT/QD conjugates were conducted. Having attributed the red shifts of some peaks of the SWCNT FTIR spectra to the transfer of electrons to the SWCNTs, we hypothesize that photoexcited conjugated QDs are likely donors of excitonic electrons to SWCNTs. This may result in positively charged QDs, which undergo more rapid photooxidation within a few milliseconds to seconds depending on the excitation power and local oxygen concentration. Consequently, QDs are expected to exhibit rapidly decreasing quantum yield, fluorescence lifetime, and emission spectrum blue shift due to the reduction of the quantum confinement length scale or localized strain in the photo-oxidized QD.

Figure 6B shows fluorescence spectra collected at different locations of a conjugate sample containing a “bundle” of DNA–SWCNTs and conjugated QDs, as shown in the fluorescence image (Figure 6A) of the sample placed on a glass substrate. Coexisting with the relatively broad fluorescence spectra of QDs are three sharp Raman peaks (Figure 6B,
To further confirm that the amide conjugation of QDs onto DNA–SWCNTs is essential to induce the blue shift, the following control experiments were conducted. First, we measured the fluorescence spectra from QDs associated with SWCNTs by physisorption after the simple mixing of QDs with SWCNT solution in the absence of the chemical reagents necessary to induce amide bonding. After precipitating the mixture, the fluorescence spectrum of a dried SWCNT–QD aggregate on a glass substrate was measured by confocal fluorescence microscopy (Figure 6C). Contrary to QDs covalently attached to DNA–SWCNTs, the blue shifts in the spectra from physisorbed QDs in this aggregate are much smaller, about 1 nm or less, indicative of substantially weaker interaction between physisorbed QDs and SWCNTs. The second control experiment investigated the influence of DNA molecules on the spectral shift of QDs. The emission spectra of small droplets of sample solutions of QDs only, DNA-conjugated QDs with no SWCNTs, and DNA–SWCNT/QD conjugates are compared in Figure 6D. The QD–DNA exhibits a negligible blue shift, which indicates that DNA conjugation to the QDs has little effect on its spectral properties, while further conjugation with SWCNTs results in a noticeable blue shift of about 4 nm or larger, which is consistent with the result for the sample where carboxyl QDs were conjugated onto DNA–SWCNTs (Figure 6B).

In summary, we have reported a new synthesis technique for the conjugation of SWCNTs and QDs using DNA molecules as linkers through an amidation reaction. A variety of optical spectra (FTIR, fluorescence emission, and UV/Vis/NIR absorbance) of QDs and SWCNTs measured prior to and after conjugation provide strong evidence that the conjugated complexes retain the good water solubility of the QDs and structural properties of the SWCNTs. Our results also support the model of electron transfer from QDs to SWCNTs, thus proving that our conjugation method results in nanoscale proximity between the QDs and SWCNTs. We believe that our technique will be of great use in a variety of real applications, such as the assembly of biological hierarchical structures, manufacture of water-soluble CNT-based composites, and fabrication of QD–CNT hybrids for nanoelectronics and nanosensors.

Experimental Section

Synthesis of DNA-wrapped SWCNT/QD complexes: Purified HiPco SWCNTs were obtained from Carbon Nanotechnologies, Inc., and used as received. The preparation of DNA-wrapped SWCNTs (DNA–SWCNTs) is described in detail elsewhere. Briefly, SWCNTs were sonicated in buffer solution (NaCl in DI water (200 mmol L⁻¹), Tris buffer (100 mmol L⁻¹), Na₃ solution (5 mmol L⁻¹) buffered to pH 7 with HCl) in the presence of 30-mer 5'-GT(GT)₁₃GT-3' single-stranded DNA, followed by centrifugation at 21 000 g for 2 h. The resulting supernatant was a stable, black liquid containing well-dispersed SWCNTs. The functionalized QDs were commercially available products from Nanomaterials and Nanofabrication Laboratories (NN-Labs) and Invitrogen. Both COOH-terminated (COOH-QD) and NH₂-terminated QDs (NH₂-QD) were used. The conjugation procedure (Figure 1) is

Figure 5. A) Typical UV/Vis/NIR absorbance spectra of the DNA–SWCNT/QD conjugates, DNA–SWCNTs, and NH₂-QDs. The spectrum of the DNA–SWCNT/QD material was taken directly from the solution after preparation; the DNA–SWCNTs sample was prepared by filtering through a 0.1-μm polycarbonate membrane and then resuspending in deionized (DI) water after several washings; the spectrum of the QDs was obtained from a 1000-fold dilution of the as-received product in DI water. Also shown is a spectrum from water. 

B) Schematic illustration of the density of states (DOS) of SWCNTs. a) Semiconducting SWCNT where S₁ and S₂ represent the first and second interband transition of the UV/Vis/NIR absorption; b) metallic SWCNT where M₁ is the first interband transition of the UV/Vis/NIR absorption. $E_F$ is the Fermi energy.

peaks on the left-hand side of the broad QD emission in spectrum b) from the DNA–SWCNTs: tangential-mode G band at 1575 cm⁻¹; disorder-induced D band at 1334 cm⁻¹; and G' band at 2661 cm⁻¹, an overtone of the D band. This coexistence of fluorescence and Raman peaks confirms that QDs and SWCNTs are physically co-localized as a result of the chemical conjugation by amide bonding described above, with further evidence of amide bonding supported by the discussion below. The fluorescence emission peaks of QDs from different sample positions a–c are at 597.3, 597.6, and 595.7 nm, respectively, which are blue-shifted (about 4–5 nm) from the 601.7 nm of QDs in a QD-only solution in the absence of DNA–SWCNTs. These blue shifts, with the average corresponding to (16.2 ± 7.7) meV, are observed from the entire sample, which implies that QDs are in nanoscale proximity to SWCNTs.
similar to that described previously by Banerjee and Wong. The SWCNTs were first dispersed in (2-N-morpholino)ethanesulfonic acid (Aldrich) buffer (0.1 mmol L\(^{-1}\)), followed by the addition of EDC (50 mmol L\(^{-1}\), Aldrich) and sulfo-NHS (100 mmol L\(^{-1}\), Fluka). For spectroscopic and confocal measurements, COOH-QDs were added dropwise to the SWCNT-suspended mixture while stirring to a final 1:1 number ratio of QDs to DNA molecules. From our FTIR results, the estimated DNA to QD molar ratio was 50:1, assuming 50 carboxyl groups per QD, but the actual concentration ratio was not measured. For the TEM experiment, a further diluted (10-\(\times\)) QD concentration was used to prevent large aggregation of SWCNTs and clustering of QDs. After stirring for 48 h at 50 °C, the solution was filtered with a 0.2-\(\mu\)-m-pore polycarbonate membrane and washed three times with DI water to remove free QDs. The product remaining on the filter was resuspended in DI water.

**Synthesis of COOH-terminated SWCNT/NH2-terminated QD complexes:** In parallel to the process above, we also synthesized SWCNT/QD complexes by reacting COOH-terminated SWCNTs (COOH-SWCNTs) and NH\(_2\)-QDs. To this end, COOH-SWCNTs were prepared by adding SWCNTs (20 mg) to HNO\(_3\) (10 mL, 3 mol L\(^{-1}\)), sonicating the solution for 2 h, and collecting COOH-SWCNTs by refluxing at 100 °C for 8 h. The harvested SWCNT solution was diluted with DI water (200 mL), centrifuged at 9400 g for 60 min, and the supernatant solution was decanted. The process of washing/centrifuging/decanting was repeated twice. The final pellet was resuspended in DI water and filtered with a 0.2-\(\mu\)-m-pore polycarbonate membrane before drying in a vacuum for 14 h.

to the measurement. For AFM measurements, a premixed solution (20\(\mu\)L) containing QDs and DNA–SWCNTs was incubated on a freshly cleaved mica substrate for 10 min, followed by several washes with ultrapure water. After drying, the sample was characterized in the tapping mode using a silicon probe (Olympus AC240) and an AFM scanner (Dimension 3100, Veeco Instruments). Fluorescence images of the QDs in the conjugates were obtained with a combined confocal fluorescence microscope and spectroscopic equipped with a 488 nm excitation laser with a photon-counting avalanche photodiode with a bandpass filter ([585 ± 35] nm) to selectively detect emission only from QDs. This confocal microscope was capable of simultaneous confocal fluorescence and Raman spectroscopic imaging to monitor the spectral shift of the QDs and to enable co-localization imaging of fluorescence from QDs and Raman shifts from SWCNTs.

**Keywords:** carbon nanotubes • charge transfer • DNA • nanocomposites • quantum dots

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