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PREPARATION AND STUDIES OF FLUORESCENT CARBON NANOMATERIALS

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PREPARATION AND STUDIES OF FLUORESCENT CARBON NANOMATERIALS

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy
Chemistry

by
Xin Wang
October 2010

Accepted by:
Dr. Sun, Ya-Ping, Committee Chair
Dr. Pennington, William T.
Dr. Dominy, Brian
Dr. Bhattacharyya, Gautam
ABSTRACT

There have been rapid advances in the development and applications of semiconductor quantum dots (QDs) represented by CdSe/ZnS. However, a serious limitation of these QDs is the necessary use of toxic heavy metals. Fluorescent “carbon dots” (surface passivated carbon nanoparticles) are developed as alternative to classical semiconductor QDs.

The carbon dots could be made to be highly fluorescence, with emission quantum yields close to 60%. Their optical properties resemble bandgap transitions found in nanoscale semiconductors, suggesting that carbon particles at the nanoscale acquire essentially semiconductor-like characteristics.

The fluorescence in carbon dots could be quenched efficiently by electron acceptor or donor molecules in solution, namely that photoexcited carbon dots are both excellent electron donors and excellent electron acceptors, thus offering new opportunities for their potential uses in light energy conversion and related applications.

Carbon dots were doped by various inorganic salts, and the spectroscopic performances of carbon dots were found to be strongly related to the types of dopants. Experiment results showed that the fluorescence brightness of carbon dots was significantly enhanced by ZnS, ZnO or TiO$_2$ as a dopant. Further fractionating the doped carbon dots resulted in some dots of the quantum yields up to 75%.

As known in the literature, well-dispersed and functionalized carbon nanotubes exhibit visible fluorescence emissions due to passivated defects on the nanotube surface. It was found in this study that the defects in nanotubes could be decorated by an
inorganic salt, which augmented the passivation effect of organic functionalization to result in dramatically enhanced emission intensities under both one- and two-photon excitation conditions. The structures and properties of the functionalized carbon nanotubes with inorganic coating were thoroughly characterized by using spectroscopy and microscopy techniques. The fluorescence decoration with the coating may serve as a tool in the study of surface defects in carbon nanotubes, and these brightly fluorescent pseudo-one-dimensional nanomaterials may be exploited for optical applications.
DEDICATION

This dissertation is dedicated to my beloved parents, Huiren Wang and Hui Li, my sister, Hongjing Wang and especially my wife, Qian Li for their love and unconditional support.
ACKNOWLEDGMENTS

I would like to express my gratitude to my advisor, Dr. Ya-Ping Sun for his wonderful guidance, his time spent with me and continued support throughout my graduate study period. His knowledge, dedication and diligence toward science have made a great impression upon me, which will guide me throughout my whole life.

Firstly, I would like to give thanks to Dr. Mohammed J. Meziani, Dr. Bing Zhou, Dr. Suyuan Xie, Dr. Kurukulasuriya A. S. Fernando, who were the first ones leading me into the beautiful scientific area and showing me the excellent scientific culture of Sun research group. I would also like to give my special thanks to Dr. Fushen Lu and Dr. Li Cao, whom I am very fortunate to work with in last five years. My thanks also go to current group members: Dr. Pengju G. Luo, Dr. Leilei Tian, Dr. Anilkumar Parambath, Dr. Changyi Kong, Ms. Jiahui Liu and Mr. Sushant P. Sahu, and the past members: Dr. Yi Lin, Dr. Liangwei Qu, Dr. Huaping Li, Dr. Lingrong Gu, Dr. Wei Wang, Dr. Lucia M. Veca, Dr. Haifang Wang, Dr. Puyu Zhang, Dr. Heting Li, Dr. Gang Qi, Dr. Bailin Chen, Dr. Pankaj Pathak, Dr. Yang Liu, Ms. Barbara Hurruff, Mr. Darron Hill and Mr. Shengtao Yang.

I am grateful to my committee members: Dr. William T. Pennington, Dr. Brian Dominy, and Dr. Gautam Bhattacharyya for their precious time and active help in the completion of this dissertation.
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CHAPTER ONE

DEVELOPMENT OF PHOTOLUMINESCENT CARBON NANOPARTICLES

1.1 Introduction

Fluorescence, first reported by Sir John Frederick William Herschel in 1845,\(^1\) is typically observed in organic dyes (aromatic molecules) such as quinine and rhodamine B. More recently, luminescent nanomaterials have attracted much attention for use in various applications because of their optical properties, including long-term stability and no blinking, which not found in organic dyes. Semiconductor quantum dots appear to be the best candidates;\(^2-7\) however their toxicity limits their potential applications.\(^8,9\) Other benign nanomaterials have also been explored for use in biological and other applications, specifically carbon-based materials such as luminescent nanodiamonds (typically > 20 nm)\(^10-12\) and luminescent carbon nanoparticles\(^13-16\) (typically < 10 nm).

1.1.1 Semiconductor Quantum Dots

With the potential to replace organic dyes, semiconductor quantum dots are currently the most promising class of fluorescent materials because of their outstanding optical properties, including size-controlled fluorescence characteristics,\(^2,17-20\) high photostability,\(^21\) high quantum yields\(^22-26\) and photoelectrochemical activities.\(^5,27-33\) While there are many kinds of semiconductor quantum dots derived from single element Si\(^34-37\) to alloys such as InP\(^38-40\), current interest is focused on the II – VI semiconductor nanocrystals, or CdSe quantum dots, as they offer significant advantages for a variety of
purposes and applications, especially for their potential uses in bio-imaging\textsuperscript{3,4,6,41-44} and in light-harvesting materials.\textsuperscript{5,27,28} The synthesis, optical properties, and applications of semiconductor quantum dots have been documented well in a number of recent reviews.\textsuperscript{3-7,27,44}

As widely acknowledged, however, the primary disadvantage of these quantum dots, especially CdSe quantum dots, is their toxicity. Because they are primarily composed of cadmium or other heavy metals, they have severe health risks.\textsuperscript{8,9,45} For example, cadmium toxicity is associated with liver and kidney damage, osteomalacia, osteoporosis, skeletal deformations, neurological problems, and other health issues.\textsuperscript{9,45} In addition, this element is considered to be a carcinogen.\textsuperscript{45} The major concern on CdSe quantum dots is the toxicity resulting from the CdSe core, especially if it is uncoated since the free cadmium present after synthesis or released from the core of quantum dots\textsuperscript{46-48} generates free radicals to “nick” DNA.\textsuperscript{49,50} In addressing this issue, researchers have found that the toxicity from semiconductor cores can be partially reduced by enclosing them in a ZnS shell or using some other capping materials.\textsuperscript{48,51} However, there are still potential and even practical issues related to safety. For example, the degradation of the ZnS shell and other capping materials\textsuperscript{52-54} can be toxic. According to the discussion in ref. 47 and 50, a ZnS shell did not completely eliminate the free radical species generated from CdSe cores. Moreover, the ZnS shell and capping materials themselves can result in toxicity issues as well.\textsuperscript{50,55} Additionally, studies in animal have indicated toxicity to vertebrate systems at relatively low concentrations\textsuperscript{47,48,57,58} and accumulation in organs and tissues.\textsuperscript{59-62}
1.1.2 Luminescent Nanodiamonds

Recently, diamond-based luminescent nanoparticles have been suggested as candidates for biological, quantum information processing, and other applications. Luminescence originates from defect sites within the diamond structure which contain impurities (non-carbon atoms). Nitrogen, the most common impurity, forms a series of color centers in the diamond structure. The Nitrogen–vacancy complex (N-V defect), which is comprised of a single nitrogen impurity in a substitutional position directly adjacent to a lattice vacancy, is one of these color centers and has attracted attention for possible use in the field of nanomedicine. The N-V defects can be efficiently created through the electron irradiation (2 or 3 MeV) of type Ib diamond nanopowders, and then thermally annealed at high temperature (700-900 °C) in vacuum. The fluorescence spectra observed (using a confocal optical microscope with a 532 nm solid-state laser as the excitation source) are highly heterogeneous, with two sharp zero-photon lines (ZPLs) representing two types of N-V defects. The first at 576 nm corresponds to the electronic transition of the neutral defect center (N-V)$^0$, and the second at 638 nm corresponds to the negatively charged defect center (N-V)$^-$. Both ZPLs are accompanied by broad phonon sidebands with a red shift of ~50 nm. The emission intensities of luminescent nanodiamonds under excitation with a 532 nm light at a power density of $8 \times 10^3$ W/cm$^2$ are very stable, showing no change over 300s, a sharp contrast to complete photobleaching found in single dye molecules (Alexa Fluor 546) within 12s. The quantum efficiency of a single defect center reported in the literature is close to 1.
These properties, combined with their nontoxic nature,\textsuperscript{10,72-76} have resulted in increasing use of luminescent nanodiamonds in biological applications such as cell imaging.\textsuperscript{10,63,65-67,70}

However, applications of luminescent nanodiamonds are limited by two disadvantages. The first is the low fluorescence intensity of individual nanodiamonds. Fu \textit{et al.}\textsuperscript{63} reported 35 nm nanodiamonds containing \textasciitilde 100 defect centers per particle showed a similar emission intensity to a single CdSe quantum dots (core size \textasciitilde 4 nm) under the same conditions. These results suggest that even if the diameter of the nanodiamonds can be decreased to 10 nm with the defect/volume ratio remaining the same, their average intensity would still be much lower (\textasciitilde 40-fold lower) than that of CdSe quantum dots. Even worse, it is also reported in ref. 78 that the probability of observing an NV defect decreases rapidly as the crystal size is reduced. These results have been confirmed by Hui \textit{et al.} who found up to 8 \pm 1 (N-V)\textsuperscript{−} defects per particle in \textasciitilde 28 nm nanodiamonds.\textsuperscript{77} The second disadvantage is the large size of the nanodiamonds. There is a concern that N-V defects may not exist when the diameter of the nanodiamond is less than 10 nm. For example, Rabeau \textit{et al.} reported no N-V defects synthesized by chemical vapor deposition in nanodiamonds of less than 40 nm.\textsuperscript{78} The theoretical calculations of Barnard and co-workers\textsuperscript{79,80} also predicted that nitrogen is metastable when the particle diameter is small. Experimental results have also shown that the photoluminescence from nanodiamonds at sizes less than 5 nm was dominated by surface defects.\textsuperscript{81} Although N-V defect centers were detected by Smith \textit{et al.} in 5 nm nanodiamonds,\textsuperscript{82} the luminescence from N-V defects was very weak in comparison to large nanodiamonds that coexisted
with and were quenched\textsuperscript{81} by surface-defect luminescence and that might unstable under annealing conditions. Thus, even though research indicates that luminescent nanodiamonds may be widely useful in future work, currently their optical performance cannot compete with that of CdSe quantum dots.

1.1.3 Luminescent Carbon Nanoparticles

Both semiconductor quantum dots and luminescent nanodiamonds exhibit promising optical properties, but with obvious limitations. Recently, another carbon-based luminescent nanoparticle has emerged as a new category of fluorescence materials which can be used in many biological applications.\textsuperscript{13-16,83,84} These carbon nanoparticles have been characterized as small,\textsuperscript{13-16} strongly fluorescent,\textsuperscript{13,85} highly photostable\textsuperscript{13,86} and non-toxic.\textsuperscript{87} Their synthesis, optical performance, and applications are discussed in sections 1.2-1.4.

1.2 Synthesis of Luminescent Carbon Nanoparticles

Recently, several methods for obtaining luminescent carbon nanoparticles were reported by Sun and other groups.\textsuperscript{13-16} One common objective of these synthetic methods involves generating carbon nanoparticles of less than 10 nm in diameter. Top-down approaches (Scheme 1.1, A) are usually used to produce carbon nanoparticles by breaking down bulky carbon materials such as graphite, multiwalled carbon nanotubes, or candle soots.\textsuperscript{13-15} Various strategies have been applied to create luminescent carbon nanoparticles, including surface passivation of laser-produced carbon soot,\textsuperscript{13}
electrochemical treatment of carbon-based materials,\textsuperscript{14,84,88} and acid treatment of candle or natural gas soot.\textsuperscript{15,89,90} Bottom-up approaches (Scheme 1.1, B) include synthesis of the luminescent carbon nanoparticles from carbonization of organic molecules.\textsuperscript{16,86,91,92}

1.2.1 Surface Passivation of Laser-Produced Carbon Nanoparticles

Since the 1980s, pulsed lasers, such as UV excimer lasers and Nd:YAG lasers, have been used for the deposition of either diamond-like or amorphous carbon films onto various substrates.\textsuperscript{93-96} Further investigation has focused on the synthesis of nanoscale carbon nanoparticles through laser pyrolysis.\textsuperscript{97-99} In those experiments, carbon targets were irradiated under such conditions as in argon gas or aqueous solution. No significant photoluminescence was reported from those laser-produced carbon nanoparticles, perhaps because of either the large size of the carbon nanoparticles (from 10 nm to 100 nm) or the lack of surface passivation.

In 2006, Sun and co-workers found that nanoscale carbon particles upon simple surface passivation (carbon dots) exhibited strong photoluminescence in both solution and the solid state\textsuperscript{13} (Scheme 1.1). These nanoscale carbon particles were first synthesized through laser pyrolysis (Nd:YAG laser, 1064 nm) of a graphitic target, and then followed by acid treatment and surface passivation with organic molecules such as PEG\textsubscript{1500N} (diamine-terminated oligomeric polyethylene glycol, \(\text{H}_2\text{NCH}_2(\text{CH}_2\text{CH}_2\text{O})_{35}\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2\)) or PPEI-EI (polypropionylethyleneimine-co-ethyleneimine). Both PEG\textsubscript{1500N}\textsuperscript{-} and PPEI-EI-functionalized samples were found to be
A). Top-down approaches

Scheme 1.1 Synthetic methods for producing luminescent carbon nanoparticles A) Top-down approaches B) Bottom-up approaches
Figure 1.1 Top: Representative STEM images of carbon dots surface-passivated with (a) PEG\textsubscript{1500N} and (b) PPEI-EI; Bottom: AFM topography (left), phase (middle), and amplitude (right) images of carbon dots with surface passivation by PPEI-EI polymers.

(From Ref. [13].)
well-dispersed and approximately 5 nm in diameter, as confirmed by both TEM and AFM analyses (Figure 1.1).

A similar method was used by Hu and co-workers, although they changed the experimental conditions and the targeting materials\textsuperscript{100,101} (Table 1.1). A Nd:YAG laser was used to irradiate dispersed graphite powders (or carbon black) in an aqueous solution, and subsequently treated with acid and surface passivation. It may also be possible to perform laser irradiation and surface passivation in one step by irradiating the graphite powders dispersed in organic solvents such as PEG\textsubscript{200N}.\textsuperscript{102}

The common consensus is that organic molecules can be attached to the surface of carbon nanoparticles. However, there is disagreement about the determination of the structures of the carbon cores. Sun and co-workers\textsuperscript{13} obtained Raman spectra (633 nm excitation) of carbon nanoparticles without passivation, representing the contributions of both sp\textsuperscript{2} (G-band at 1,590 cm\textsuperscript{-1}) and sp\textsuperscript{3} carbons (D-band at 1,320 cm\textsuperscript{-1}). However, this result cannot be used to determine the structure of carbon cores since both multi-layer graphene and nanodiamonds at 5 nm in size exhibit similar Raman spectra.\textsuperscript{82,103,104} The structure of carbon cores was described as amorphous carbon since no clear crystalline structures were visualized in HR-TEM images.\textsuperscript{85} Other research groups\textsuperscript{101-102} claimed that nanodiamond structures were formed by laser irradiation on the basis of HRTEM images of single nanoparticles and electron diffractions, which was questionable since the lattice fringes of the diffraction planes of diamond-like and graphitic carbons are very close to each other.\textsuperscript{89} Further experimental evidence such as $^{13}$C NMR spectra of carbon dots are needed to verify the structures of carbon cores.
Table 1.1 Structure and optical parameters of carbon nanoparticles synthesized using various methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Starting Materials</th>
<th>Particle Size</th>
<th>Core Structure</th>
<th>Quantum Yields (Wavelength)</th>
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<tr>
<td>Laser ablation</td>
<td>Graphite</td>
<td>~ 5 nm</td>
<td>Amorphous carbon</td>
<td>4-10% (400nm)</td>
<td>13</td>
</tr>
<tr>
<td>Surface passivation</td>
<td>Graphite</td>
<td>~ 5 nm</td>
<td>Amorphous carbon</td>
<td>~ 50% (440nm)</td>
<td>85</td>
</tr>
<tr>
<td>Laser ablation in water</td>
<td>Graphite</td>
<td>~ 7.2 nm</td>
<td>Diamond</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Surface passivation</td>
<td>Graphite</td>
<td>~ 3 nm</td>
<td>Diamond</td>
<td>3-8%</td>
<td>101</td>
</tr>
<tr>
<td>Surface passivation</td>
<td>Carbon black</td>
<td>~ 4 nm</td>
<td>Diamond</td>
<td></td>
<td>102</td>
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<tr>
<td>Electrochemical treatment</td>
<td>MWCNTs</td>
<td>2.8 ± 0.5 nm</td>
<td>Graphite</td>
<td>6.3% (340nm)</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Graphite</td>
<td>1.9 ± 0.3 nm</td>
<td>Graphite</td>
<td>1.2% (330 nm)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.2 ± 0.5 nm</td>
<td></td>
<td></td>
<td></td>
<td>84,88</td>
</tr>
<tr>
<td>Acid treatment of carbon soots</td>
<td>Candle soot</td>
<td>~ 1 nm</td>
<td></td>
<td>~ 1% (366 nm)</td>
<td>15</td>
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<tr>
<td></td>
<td>Natural gas soot</td>
<td>4.8 ± 0.6 nm</td>
<td>Graphite</td>
<td>0.43% (310 nm)</td>
<td>89</td>
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<tr>
<td></td>
<td>Candle soot</td>
<td>2-6 nm</td>
<td>Graphite</td>
<td>~3%</td>
<td>90</td>
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<tr>
<td>Pyrolysis</td>
<td>Amino organic</td>
<td>~ 7 nm</td>
<td>Graphite oxide</td>
<td>3% (495 nm) (340 nm)</td>
<td>16, 91</td>
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<tr>
<td></td>
<td>molecules</td>
<td></td>
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<tr>
<td>Dehydration</td>
<td>Carbohydrates</td>
<td>~ 5 nm</td>
<td>Graphite oxide</td>
<td>13% (360 nm)</td>
<td>112</td>
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<tr>
<td>Surface passivation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Microwave</td>
<td>Saccharide</td>
<td>2.75 ± 0.45 nm</td>
<td>Amorphous carbon</td>
<td>3.1%-6.3%</td>
<td>92</td>
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<tr>
<td>Surface passivation</td>
<td>3.65 ± 0.6 nm</td>
<td></td>
<td></td>
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<tr>
<td>Pyrolysis and Etching</td>
<td>Resol/F127/SiO2</td>
<td>1.5-2.5 nm</td>
<td>Amorphous carbon</td>
<td>14.7% (360 nm)</td>
<td>86</td>
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<td>composites</td>
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In addition, it is important to remember that the structures of laser-produced carbon nanoparticles can vary based on such laser irradiation conditions as the furnace temperature or the laser intensity. It is also possible different structures of carbon cores can be formed under various laser irradiation conditions.

### 1.2.2 Electrochemical Treatment of Carbon-based Materials

Another effective method for making luminescent carbon nanoparticles is electrochemical decomposition/oxidation of carbon-based materials (Scheme 1.1). In 2007, Zhou et al. first reported that the electrochemical treatment of multwall carbon nanotubes (MWCNTs) could yield blue luminescent graphitic nanocrystals. In their experiments, MWCNTs deposited on carbon paper were used as a working electrode, while Pt wire and Ag/AgClO4 functioned as the counter electrode and reference electrode, respectively. The applied potential at the working electrode was cycled between 2.0 V and 2.0 V at a scan rate of 0.5 V/s. Graphitic nanocrystals with a small distribution range of 2.8 ± 0.5 nm were generated and dispersed into electrolyte solutions (TBAP) along with the breaking process of MWCNTs during the electrochemical cycling (Figure 1.2, Top). The surface chemical components of these carbon nanocrystals were examined using X-ray absorption near-edge structure (XANES) and X-ray excited optical luminescence (XEOL) to show the surface impurities (vacancies) of O and N elements, which may be essential to the mechanism of luminescence. A similar electrochemical treatment has also been applied to the
oxidation of graphite.\textsuperscript{84,88} Zhao et al., for example, reported a graphite column electrode (working electrode) could be electro-oxidized to nano-sized carbon graphitic crystals which were separated by weight cutoff membranes. According to the TEM results (Figure 1.2, bottom), the first two fractions with molecular weights $> 5$ and 5-10 kDa had a spherical shape and a small distribution range of $1.9 \pm 0.3$ nm and $3.2 \pm 0.5$ nm, respectively.

1.2.3 Acid Treatment of Candle or Natural Gas Soot

Luminescent carbon nanoparticles were also synthesized through either the acid treatment of candle or natural gas soot.\textsuperscript{15,89} Candle or natural gas soot was first collected from a glass plate placed over a smoldering candle or a beaker placed upside-down above the flame of a natural gas burner and then followed by refluxing in 5 M HNO\textsubscript{3} for 12 h to generate luminescent carbon nanoparticles (Scheme 1.1). AFM (Figure 1.3) and elemental analysis determined that the luminescent carbon nanoparticles from candle soot were quite small, approximating 1 nm with a 10 atom\% nitrogen content.\textsuperscript{15} The size disparity of the carbon nanoparticles from natural gas soot was from 4.4 to 5.4 nm (Figure 1.3), larger than that from candle soot. HR-TEM images indicated that the lattice structures of carbon cores were identical to graphitic carbon, which was also confirmed by $^{13}$C NMR results.\textsuperscript{89} The peaks of the $^{13}$C NMR spectrum were only observed within the range of 120 to 150 ppm and 170 to 180 ppm, indicating aromatic carbon cores and peripheral carboxylic/carbonyl carbons. The fact that no signal was seen below 120 ppm indicated no diamond-like carbon structures were found.
Figure 1.2 TEM images and HRTEM images (Inset) of luminescent nanocrystals synthesized by electrochemical treatment of MWNTs (Top) and Graphite (Bottom (a) carbon nanocrystals <5kDa, (b) carbon nanocrystals 5-10kDa) (From Ref. [14] and [88].)
An additional efficient separation method, recently proposed by Ray et al., involves selectively obtaining carbon nanoparticles from acid-treated candle soot. Since carbon nanoparticles generated from acid-treated candle soot are very stable when suspended in either aqueous or ethanolic solutions even at 16,000 rpm centrifugation, carbon nanoparticles were dissolved in a solvent mixture of water-ethanol-chloroform (volume ratio 1:1:3) as centrifugation precursor, the chloroform being specifically targeted to decrease the solubility of carbon nanoparticles. The supernatant collected after 8,000 rpm centrifugation contained graphitic carbon particles 2-6 nm in diameter composed of 4 atom% nitrogen and 37 atom% oxygen.

1.2.4 Carbonization of Organic Molecules

Although recent research on synthesizing luminescent carbon nanoparticles has focused on top-down strategies, there has also been some work on the bottom-up approach. For example, though Kowalewski and co-workers synthesized carbon nanoparticles approximating 10 nm or smaller through pyrolysis of organic compounds solubilized either in organic solvents or in an aqueous solution, no luminescence was reportedly exhibited by those carbon nanoparticles. Bourlinos et al. were the first to report observing luminescence from carbon nanoparticles synthesized by thermal decomposition (carbonization) of either ammonium citrate salts or 4-aminoantipyrine in air at 300 ºC (Scheme 1.1). The structure of carbon nanoparticles made from ammonium citrate salts was characterized by HR-TEM and XRD patterning. The images
Figure 1.3 Top: A representative AFM image of luminescent carbon nanoparticles derived from candle soot; Bottom: Representative TEM micrographs of carbon nanoparticles derived from natural gas at (A) low and (B) high resolution. (From Ref. [15] and [89].)
Figure 1.4 Top: TEM (left) and HRTEM images of carbon nanoparticles synthesized through pyrolysis of citrate salt. The corresponding insets show individual core-shell dots and the SAED pattern of the crystalline core. The HRTEM images are from the analysis of the inset particle shown with the arrow; Bottom: core size histogram of carbon nanoparticles. (From Ref. [91].)
showed that both aromatic and aliphatic regions were found in the carbon cores ranging from 5 – 10 nm size and the structures were most likely similar to graphite oxide (Figure 1.4). In addition to direct thermal decomposition, other applied carbonization methods include microwave synthesis, the use of silica spheres as carriers for carbon nanoparticles, and dehydration of carbohydrates (Scheme 1.1).

1.2.5 Luminescent Carbon Nanocomposites

In 2008, Sun and co-workers reported that semiconductor doping on the surface of carbon dots ($C_{ZnS}$-dots or $C_{ZnO}$-dots) could achieve much higher photoluminescence quantum yields than undoped carbon dots, which is comparable to the commercially available CdSe/ZnS quantum dots. In their experiments, semiconductors such as ZnS or ZnO were doped on the surface of acid-treated carbon nanoparticles, and followed by SDS activation and surface passivation. Carbon dots at approximating 5 nm were visualized by high-resolution TEM which showed partially doped semiconductors on the carbon surface (Figure 1.5, Top). In addition to semiconductors, it is also possible to deposit metals such as Ag, Cu and Pd on the surface of carbon particles to form metal-carbon nanocomposites through chemical reduction. Metal ions were mixed experimentally with carbon nanoparticles produced by acid treatment of natural gas soot in an aqueous solution and then reduced using ascorbic acid. The TEM images indicated nanocomposites with an average diameter of 16-20 nm (Figure 1.5, Bottom) were composed of the metal particles embedded in the carbon matrix. After deposition, the metal-carbon nanocomposites exhibited photoluminescence with a slight red shift.
1.3 Photoluminescence Properties of Carbon Nanoparticles

Luminescent carbon nanoparticles have the potential as an alternative to semiconductor quantum dots because their optical performance is as good as or even better than that of quantum dots, including strong photoluminescence (high quantum yields and absorptivities),\textsuperscript{13,85,107} high photostability (low photobleaching and no blinking)\textsuperscript{13,86,88} and strong two-photon emission.\textsuperscript{108} However, there are still many questions related to these carbon nanoparticles that must be resolved, primarily about their luminescence mechanism. Several research groups have proposed luminescence mechanisms individually based on observations of the behavior of carbon nanoparticles, which are mostly conflicted with each other. Sections 1.3.1 to 1.3.4 discuss these issues.

1.3.1 Luminescence Brightness, Photostability and Two-photon emission of Carbon Nanoparticles

Fluorescence brightness is proportional to the quantum yield and molar absorptivity of the fluorophore. Table 1.1 shows that surface passivation is an essential step for acquiring high quantum yields, regardless of carbon sources and preparation methods. Without surface passivation, quantum yields of most naked carbon nanoparticles are much less than 5%.\textsuperscript{15,16,84,88,89,90,91} A clear demonstration of the effects of surface passivation was reported by Peng \textit{et al.}, who prepared carbogenic dots through the dehydration of carbohydrates such as glucose and followed by passivation with 4,7,10-trioxa-1,13-tridecanediamine (TTDDA). The results indicated that TTDDA
Figure 1.5 Top: A representative TEM (Z-contrast) image and HR-TEM image (Inset) of C\textsubscript{ZnS}-Dots; Bottom: Representative TEM (left), HR-TEM (right) and histogram (Inset) of carbon nanoparticles functionalized with silver. (From Ref. [85] and [89].)
passivation dramatically enhanced the photoluminescence intensity of carbogenic dots, quantum yield improving from 1% to 13%, while the emission spectra with a slightly narrower bandwidth did not significantly shift after surface passivation. Quantum yields of carbon nanoparticles vary, depending on the types of surface-passivation, with most being near to or higher than 10%, comparable to luminescent Si nanoparticles. Until recently, the maximum of quantum yields reached 57% for carbon nanoparticles and up to 75% (at 440 nm excitation) for doped carbon nanoparticles. These quantum yields are competitive to or even higher than those in commercially available CdSe/ZnS quantum dots, indicating their potential usefulness in a myriad of applications.

In addition to quantum yields, the high absorptivities of carbon nanoparticles make them more promising for further use. The radiative rate constant ($k_F$) of separated carbon dots with a 57% quantum yield was measured by Sun and co-workers, approximating 3 times higher than that in commercially available CdSe/ZnS quantum dots. Since radiative rate constants are proportional to integrated molar absorptivities, the luminescence of carbon dots at the single molecular level can be much brighter than that of quantum dots. This conclusion was confirmed by confocal fluorescence imaging results.

The photostability including low photobleaching and no blinking is another significant facet in the research of luminescent carbon nanoparticles. Sun and other groups found that the photoluminescence of surface-passivated carbon dots caused no meaningful reduction in the observed intensities after continuously repeating excitations for several hours. They also found no blinking in the luminescence emissions of these
carbon dots (Figure 1.6, Top), unlike in organic dyes, CdSe quantum dots and other nanoparticles.\textsuperscript{13,86} Similarly, Zhao \textit{et al.}\textsuperscript{88} demonstrated the stability of photoluminescence of carbon nanoparticles synthesized through electronchemical treatment of graphite after a continuous excitation over 6 h with a Xe lamp (8.3 W) (Figure 1.6, Top). These investigations indicate the superiority of carbon nanoparticles over organic dyes and most semiconductor quantum dots, suggesting their applications in single molecular tracking and for long-term \textit{in vitro} and \textit{in vivo} observations.

Surface-passivated carbon dots also exhibit very strong two-photon activity.\textsuperscript{108} Figure 1.6 (a) (b) show one- and two-photon luminescence images under laser excitation at 458 nm and 800 nm, respectively. As compared in Figure 1.6 (c), the one- and two-photon luminescence images for the same scanning area match well. The estimated two-photon absorption cross-section of carbon dots at 800 nm was $39,000 \pm 5,000$ GM, the same level as the two-photon absorption cross-section of CdSe/ZnS quantum dots which was estimated to be on the order of $50,000$ GM.\textsuperscript{41} In addition, the carbon dots were photostable under the two-photon imaging conditions with more than 3000 scanning times of repeated 800 nm excitations. Applications based on the two-photon performance of carbon dots will be discussed in section 1.4.1.

\section*{1.3.2 Excitation Wavelength Dependent Photoluminescence}

In 2006, Sun and co-workers first reported that the photoluminescence spectra of surface-passivated carbon dots are dependent on the excitation wavelength.\textsuperscript{13} Their results also showed that the emission bands continuously shifted to the red, which were
Figure 1.6 Top, left: The time-dependence of luminescence intensity of PEG$_{1500}$N-carbon dots measured in confocal microscopy (Leica TCS SP2, the frame rate 37 ms/frame at 514 nm excitation). Shown in the inset is a comparison of the same data (blue) with that of a commercially available (Ted Pella, Inc, diameter ~ 50 nm) blinking gold nanoparticles sample (red); Top, right: Dependence of fluorescence intensity on excitation time for carbon nanocrystals in ultra pure water; Bottom: Luminescence images (all scale bars 20 µm) of the carbon dots with (a) argon ion laser excitation at 458 nm and (b) femtosecond pulsed laser excitation at 800 nm; (c) is an overlay of (a) and (b). (From Ref. [13], [88] and [108].)
almost across the whole visible spectral range, and extended into the near-infrared when longer excitation wavelengths were applied (Figure 1.7). Strong photoluminescence was observed, and the fluorescence quantum yields at 400 nm excitation wavelength reached more than 10% (Table 1.1). Similar photoluminescence behavior was also reported from other groups using various synthetic methods. For example, graphitic nanocrystals produced by electrochemical treatment of multwall carbon nanotubes also exhibited this photoluminescence property. Furthermore, it is worth noting that all the luminescent nanoparticles which are currently generated from bottom-up methods also exhibited excitation wavelength dependent photoluminescence. Quantum yields of these samples are from 3% to 14.1% (Table 1.1) depending on the excitation wavelengths and the carbon sources, were comparable to those of the surface-passivated carbon dots synthesized by Sun and co-workers.

1.3.3 Bandgap-like Luminescence

In addition to excitation wavelength dependent photoluminescence, a few groups also reported that for luminescent carbon nanoparticles synthesized using specific methods, the particle-related photoluminescence spectra might not shift with varying excitation wavelengths. In 2007, Liu et al. reported that carbon nanoparticles derived from candle soot might be separated by denaturing polyacrylamide gel electrophoresis (PAGE). Separation is dependent on the physical properties of carbon nanoparticles, particularly their charges or diameters. The results showed that rapidly moving particles exhibited shorter-wavelength emissions under UV light excitation (312 nm), while slowly
Figure 1.7 Top: aqueous solution of PEG\textsubscript{1500N}-attached carbon dots (a) excited at 400 nm and photographed through band-pass filters of different wavelengths as indicated, and (b) excited at the indicated wavelengths and photographed directly; Bottom: The absorption (ABS) and luminescence emission spectra (with progressively longer excitation wavelengths from 400 nm on the left in 20 nm increment) of PPEI-EI carbon dots in an aqueous solution. The emission spectral intensities are normalized to quantum yields (normalized to spectral peaks in the inset). (From Ref. [13].)
moving particles exhibited longer-wavelength emissions under the same condition (Figure 1.8). In addition, the emission spectra were almost across the whole visible range.

In 2008, Sun and co-workers found the absorption spectra of carbon dots doped with semiconductors such as ZnS or ZnO on the surface featured a shoulder in the blue region. Strong bluish green luminescence emission was observed when excitation wavelengths were in the absorption shoulder range.\(^8^5\) This emission peak appeared with the excitation wavelength dependent photoluminescence peaks together when excitation wavelengths were focused in the blue region. Further investigation indicated that the absorption spectra of undoped carbon dots might also exhibit an absorption shoulder in the same region if experimental conditions such as temperature and moisture were well controlled.\(^1^0^7\) It is evident that the cause of this absorption shoulder is from the carbon nanoparticle itself, not from doping materials, which can be enhanced by semiconductor doping. The observed quantum yields could reach more than 50\% at 440 nm excitation (Table 1.1). Similar optical behavior was also observed by other research groups. Zhao \textit{et al.},\(^8^8\) for example, found carbon nanocrystals released electrochemically from graphite might be separated by molecular weight cutoff membranes to exhibit size-dependent luminescence spectra. Similar to those in the quantum dots, the luminescence emission spectra shifted from 445 nm to 510 nm when the diameters of the carbon nanocrystals increased from 1.9 nm to 3.2 nm (Figure 1.8). However, the emission peaks did not shift with various excitation wavelengths, which was confirmed by Chi and co-workers.\(^8^4\)
Figure 1.8 Left: Optical characterization of the purified carbon nanoparticles. Optical images illuminated under white (top) and UV light (312 nm; center), fluorescence emission spectra (excitation at 315 nm) of the corresponding carbon nanoparticle solutions. The maximum emission wavelengths are indicated above the spectra; Right: UV-vis absorption and fluorescence spectrum of < 5 kDa fraction in aqueous solution (Top). The emission spectrum was obtained under an excitation of 330 nm, and the excitation spectrum was obtained at the maximum emission wavelength of 445 nm. Inset: digital photo for the product, illuminated with a UV lamp; Fluorescence spectrum of 5–10 kDa fraction in aqueous solution (Bottom), excitation wavelength: 370 nm; the excitation spectrum collected at 510 nm. Inset: digital photo for the product, illuminated with a UV lamp. (From Ref. [15] and [88].)
1.3.4 Investigation of the Photoluminescence Mechanism of Luminescent Carbon Nanoparticles

Even though many different types of luminescent carbon nanoparticles were synthesized using various methods over the past four years, the photoluminescence mechanism is still unclear. What are the fluorescence species? Why does the luminescence spectra sometimes shift with various excitation wavelengths and sometimes not? Why is there such variation in the luminescence quantum yields, from less than 1% to more than 50%? These questions are very important for the photoluminescence exploration of carbon dots.

Sun and co-workers proposed that excitation wavelength dependent photoluminescence might be attributable to the presence of surface energy traps (surface defects) which could be passivated by organic molecules.\textsuperscript{13} Their results showed carbon dots with smaller diameters and better surface passivation to be the most luminescent species. Zhou et al. proposed that N-associated defects might be responsible for luminescence. Using X-ray absorption near-edge structure (XANES) and X-ray excited optical luminescence (XEOL) to compare the electric structures between luminescence of carbon nanocrystals and diamond particles,\textsuperscript{105} they found that N incorporated into the sp\textsuperscript{2} carbon structures were the original species in both surface of carbon nanocrystals and diamond particles for luminescence emission. A similar explanation was used to explain the photoluminescence emanating from small-diameter nanodiamonds. For example, the photoluminescence of nanodiamonds with 5 nm in diameter was dominated by H3 defect
(nitrogen vacancy) centers which is also dependent upon wavelength excitation.\textsuperscript{82} However, Zhu \textit{et al.} reported that carbon nanoparticles synthesized by microwave pyrolysis of sacharide and poly(ethylene glycol) (PEG-200) also exhibited excitation wavelength dependent photoluminescence.\textsuperscript{92} No nitrogen atoms were involved except the N\textsubscript{2} gas in the air. Currently, although surface defects of carbon nanoparticles appear to be the key for elucidating excitation wavelength dependant photoluminescence, it is still not clear as to which of the non-carbon atoms are involved in those defects. N and O are the two most probable atoms since they can be found in most of luminescent carbon nanoparticles.

Two possible mechanisms for the bandgap-like luminescence of carbon nanoparticles have been proposed. Some hypothesize that the polyaromatic conjugation system is responsible,\textsuperscript{88} while others postulate that it may be a result of the band gaps of carbon cores.\textsuperscript{84} However, neither proposed mechanism explains all the experimental phenomena. For example, carbon nanoparticles derived from candle soot refluxed in highly oxidative acid for a lengthy period, makes it difficult to infer that the polyaromatic conjugation system can exist after such harsh treatment.\textsuperscript{15} Neither can it explain why semiconductor doping enhances the photoluminescence. In addition, the photostability of luminescent carbon nanoparticles is much higher than polyaromatic conjugated organic dyes. It is also difficult to believe carbon is a semiconductor since no research results or predictions suggest that the bandgap of either carbon nanoparticles or carbon related materials is between 1 and 4 eV. It is, thus, difficult to attribute the photoluminescence from carbon nanoparticles to the bandgap luminescence from a semiconductor.
Further, when discussing the photoluminescence mechanism, it is also important to consider the relationship between the excitation wavelength dependent photoluminescence and the bandgap-like luminescence. Are they independent or related? They may well coexist in the emission spectra of single kind of surface-passivated carbon nanoparticles. However, thus far, there is no obvious evidence indicating a relationship between these two behaviors. Photoluminescence emissions from carbon nanoparticles generated through electrochemical treatment of MWNTs and graphite showed very different spectra, excitation wavelength dependent photoluminescence and bandgap-like photoluminescence, respectively.\textsuperscript{14,88} However, both types of carbon nanoparticles were reported as graphitic nanocrystalline structures. A careful inspection of the two experimental conditions indicates that one nitrogen-contained compound (TBAP) was used in electrochemical treatment of MWNTs, but not in the other. Zhou \textit{et al.} also claimed in ref. 14 that TBAP played a role in the electrochemical process. Whether this finding indicates that either nitrogen or oxygen evolved the surface defects, which may quench bandgap-like photoluminescence needs further investigation.

1.4 Application of Luminescent Carbon Nanoparticles

1.4.1 Toxicity Studies and Biological Applications

Carbon, which forms the skeletons of millions of organic compounds and human bodies, is hardly considered to be an environmentally toxic element. However, the toxicity of carbon nanoparticles \textit{in vitro} and \textit{in vivo} must be determined before they can
be used in any biological applications, especially carbon nanotubes, another carbon-based nanostructure that has been reported in the literature as being potentially toxic. Indeed, there are only a few current publications which discuss the biocompatibility of carbon nanoparticles. Our preliminary studies showed that carbon nanoparticles are non-toxic both \textit{in vitro} and \textit{in vivo}. The cytotoxicity of luminescent carbon nanoparticles synthesized through electrochemical treatment of graphite was discerned through the MTT assay. This mixing of nanoparticles with $8 \times 10^3$ 293T human kidney cells in culture medium did not significantly affect the cell viability. Additional results supporting low cytotoxicity evaluations were reported using PEG$_{1500N}$ surfaced passivated carbon dots, based upon their effects on the proliferation, mortality and viability of human breast cancer MCF-7 and human colorectal adenocarcinoma HT-29 cells. As shown in Figure 1.9, all of three parameters exhibited very little difference among carbon dots treated with the two types of cells and PEG$_{1500N}$ itself under the same experimental conditions. These results demonstrated that the cores of carbon nanoparticles, similar to nanodiamonds, were inert and exhibited no chemical activity. However, passivation agents may induce cytotoxicity. For example, high concentrations of PEG$_{1500N}$ might be toxic to cells, perhaps limiting the usage of PEG$_{1500N}$ passivated carbon dots for cell imaging, even though high concentrations of carbon nanoparticles may not be necessary most of the time. Recently, toxicity issues of carbon dots \textit{in vivo} were also investigated. Toxicity evaluations \textit{in vivo} have been divided into two parts. The first component is represented as serum biochemistry assays, especially assays exhibiting potential heptic injury and kidney functions. As shown in
Figure 1.9 Results from cytotoxicity evaluations of carbon dots (black) and PEG$_{1500N}$(white). Data presented as mean ± SD (n = 4). (From Ref. [87].)
Figure 1.10, of the five indicators, alanine amino transferase (ALT), aspartate amino transferase (AST), uric acid (UA), blood urea nitrogen (BUN) and creatinine (Cr), the first two indicated hepatic injury, and the last three exhibited kidney functions, maintained at similar levels for those mice exposed to different dosages of carbon dots and for the control group. The results suggested no toxicity from the carbon dots in mice at exposure levels. The second component is the histopathological analyses of mice organs. 40 mg carbon core-equivalent/kg body weight of PEG\textsubscript{1500N} passivated carbon dots were injected into mice which were harvested for histopathological analyses. As shown in Figure 1.11, no structural damage was found in mice organs, including livers, spleens and kidneys after 28 days. Fluorescence images (two-photon excitation at 800 nm) suggested that the amounts of carbon dots in the liver and spleen 6 h from the time of injection were relatively higher than those in other organs (Figure 1.11); results determined amounts on the order of 20 µg and 2µg, respectively, according to the isotope analyses of PEG\textsubscript{1500N} passivated \textsuperscript{13}C dots in the organs. While these findings suggest small amounts of carbon dots accumulated in both the liver and the spleen, they were low in absolute populations which is consistent with the results in the literature as PEGylated nanoparticles are primarily excreted via urine.\textsuperscript{59}

While these results suggest that carbon nanoparticles are, to a certain degree, non-toxic both \textit{in vitro} and \textit{in vivo}, further studies are needed (e.g. genotoxicity, metabolism and long-term toxicity of luminescent nanoparticles) before their applications can be of practical use.
Figure 1.10 Serum biochemistry results for mice intravenously exposed to C-Dots at carbon core-equivalent of 8 mg/kg (gray) and 40 mg/kg (white) and the control mice (black) at 1 day (top), 7 days (middle), and 28 days (bottom) postexposure. Data presented as mean ±SD (n = 5). (From Ref. [87].)
Figure 1.11 Top: Results from histopathological analyses of liver, spleen, and kidneys. 

Bottom: Fluorescence images (two-photon excitation at 800 nm) of sliced liver and spleen harvested from mice 6 h after intravenous exposure to carbon dots. (From Ref. [87].)
There have been several reports of successful cell imaging using luminescent carbon nanoparticles. Figures 1.12 and 1.13 show confocal microscopy images of E. coli ATCC 25922 cells labeled with PEG\textsubscript{1500N} passivated carbon dots synthesized either using laser ablation\textsuperscript{13} or silica spheres as carriers\textsuperscript{86} under various excitation wavelengths. These results suggest that the E coli cells were completely covered by luminescent carbon dots which were clearly observable under confocal microsocopy imaging. Moreover, PPEI-EI passivated carbon dots were able to label both the cell membrane and the cytoplasm of MCF-7 without reaching the nucleus at 37 °C as can be seen in Figure 1.14 under the two-photon excitation condition.\textsuperscript{108} These results also suggest that the cellular uptake of carbon dots was temperature-dependent since no meaningful internalization was observed at 4 °C. An understanding of the internalization mechanism is still under investigation.\textsuperscript{108}

Furthermore, the surface passivated carbon dots were applied to optical imaging \textit{in vivo}. As reported in ref. 83, PEG\textsubscript{1500N} passivated carbon dots and ZnS doped carbon dots were used for subcutaneous injections, migration tracking through lymph vessels, and intravenous injections. Subcutaneous injections were used on female DBA/1 mice which were shaved in the rear area surrounding the injection point. Figure 1.15 shows the strong green or red luminescence emission at the injection area under blue and green excitation, respectively, which is consistent with the luminescence results of surface passivated carbon dots reported in the solution phase.\textsuperscript{13,85} PEG\textsubscript{1500N} passivated ZnS doped carbon dots were also injected into mice paws to track the migration of carbon nanoparticles through the lymph vessels. In contrast to the CdSe/ZnS quantum dots,\textsuperscript{110}
Figure 1.12 Confocal microscopy images of E. coli ATCC 25922 cells labeled with the carbon dots prepared by laser ablation: (a) $\lambda_{EX}=458$ nm, detected with a 475 nm long pass filter; (b) $\lambda_{EX}=477$ nm, detected with a 505 nm long pass filter; (c) $\lambda_{EX}=488$ nm, detected with a 530 nm long pass filter; (d) $\lambda_{EX}=514$ nm, detected with a 560 nm long pass filter. (From Ref. [13].)
Figure 1.13 Confocal microscopy images of E. coli ATCC 25922 cells labeled with the carbon dots prepared by silica spheres as carriers. A) $\lambda_{\text{EX}}=458$ nm, detected with 475 nm long-pass filter; B) $\lambda_{\text{EX}}=488$ nm, detected with 505 nm long-pass filter; C) $\lambda_{\text{EX}}=514$ nm, detected with 530 nm long-pass filter. (From Ref. [86].)
Figure 1.14 Representative two-photon luminescence images (800 nm excitation) of human breast cancer MCF-7 cells with internalized C-Dots. (From Ref. [108].)
the results suggest that carbon dots moved more slowly, perhaps due to the smaller size and surface functionalities of the carbon nanoparticles. Carbon dots were intravenously injected into mice for whole-body circulation as well. As shown in Figure 1.16, only emissions from the bladder area were clearly observed, suggesting the intravenously injected carbon dots were primarily excreted via urine.

1.4.2 Photoelectric Applications

Wang et al. reported that surface passivated carbon dots could be quenched by either electron donors or electron acceptors, indicating that the photoluminescence emission mechanism in carbon nanoparticles may be due to the radiative recombination of surface-trapped electrons and holes.111 These photoinduced redox properties of carbon nanoparticles suggest a new area of potential applications in devices such as light harvesting and light-emitting diodes. Electron activities of carbon nanoparticles obtained from natural gas were also quantified based on cyclic voltammograms in a water solution of 0.1 M KCl (PH ¬≈5) within the potential range of -1.0V to +1.0V at varied potential sweep rates.89 Most importantly, Zheng et al. reported the observation of electrochemiluminescence (ECL) from carbon nanoparticles synthesized by electrochemical treatment of graphite.84 In their experiments, the preparation of carbon nanoparticles was accomplished in an electrochemical cell consisting of a graphite rod working electrode, a Pt mesh counter electrode, an Ag/AgCl reference electrode and a pH 7.0 phosphate buffer solution. During the potential scan between -3.0 and 3.0V, weak and much stronger ECL signals were observed in the anodic (+1.5 to 3.0V) and cathodic (-1.0
Figure 1.15 Subcutaneous injection of (top) carbon dots and (bottom) C$_{\text{ZnS}}$-Dots: (a) bright field, (b, d) as-detected fluorescence (excitation/emission wavelengths indicated), and (c, e) color-coded images. (From Ref. [83].)
Figure 1.16 Intravenous injection of carbon dots: (a) bright field, (b) as-detected fluorescence (Bl, bladder; Ur, urine), and (c) color-coded images. (From Ref. [83].)
to -3.0V) potential ranges, respectively (Figure 1.17). After the solution was untrafiltrated using a 10 kDa molecular weight cutoff membrane, spherically shaped carbon nanoparticles were found to be responsible for the ECL. Further studies of ECL emissions from carbon nanoparticles\textsuperscript{84} in the presence of S\textsubscript{2}O\textsubscript{8}\textsuperscript{2-} and carbon nanoparticles obtained via the microwave method were found to be relatively stable\textsuperscript{92} (Figure 1.18).

1.5 Outline of Dissertation

Luminescent carbon nanoparticles (carbon dots), first discovered by Sun and co-workers in 2006, have attracted much attention because of their unique optical performance. Subsequent research has focused on the synthesis and spectroscopic characterization of highly fluorescent carbon dots, which can potentially be applied to many fields of research. In addition, highly fluorescent doped carbon nanotubes have also been synthesized and characterized for the mechanistical investigation of both defect site-derived luminescence and optical applications.

The research reported here is divided into the following chapters. Chapter II focuses on the investigation of the photoinduced electron transfer between carbon dots and other molecules. In chapter III, as-produced carbon dots were fractionated by a gel column to obtain carbon dots with emission yields close to 60%. Chapter IV is divided into two sections. In the first section, carbon nanoparticles were doped with inorganic salts such as ZnO or ZnS before their surface passivation with organic molecules to achieve higher photoluminescence quantum yields. In the second section, other inorganic salts, TiO\textsubscript{2}, SiO\textsubscript{2}, AgCl and Fe\textsubscript{3}O\textsubscript{4}, were applied to the dope carbon surface as the
Figure 1.17 ECL responses obtained on a GR electrode in 0.1 M PBS (pH 7.0). The applied potential was cycled between -3.0 and 3.0 V at a scan rate of 0.1 V/s. Inset: plot of ECL intensity vs. number of potential scan cycles. (From Ref. [84].)
Figure 1.18 Top: ECL of CNCs in aqueous 0.1 M PBS solution (pH 7.0) in the (a) presence and (b) absence of 1 mM K$_2$S$_2$O$_8$. Inset: ECL responses of CNCs/S$_2$O$_8^{2-}$ obtained during a continuous potential scan at 0.1 V/s. Bottom: Represenstive ECL response (a) without and (b) with CNPs at an ITO electrode in 0.1 M PBS (pH 7.0). Inset: anodic ECL response during a continuous potential scan, v = 0.1 V/s. (From Ref. [84] and [92].)
optical performances of carbon dots are strongly related to doping materials. Further fractionation of these doped carbon dots showed that quantum yields of the most fluorescent fractions reached up to 75%. Chapter V focuses on defect site-decorated carbon nanotube for mechanistical investigation and optical applications.

Chapter II and Chapter III, the first section of Chapter IV and Chapter V have been published in the literature as references 111, 107, 85, 113, respectively.
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CHAPTER TWO
PHOTOINDUCED ELECTRON TRANSFERS WITH CARBON DOTS

2.1 Introduction

Quantum-sized semiconductor nanoparticles (quantum dots) have emerged as an important class of photoactive nanomaterials for a variety of purposes and applications.\textsuperscript{1-4} For the utilization of semiconductor quantum dots in light energy conversion and related areas, there have been extensive investigations on their photoresponse and photoinduced charge separation and electron transfer processes.\textsuperscript{5-8} Alternative to the traditional semiconductors, other quantum-sized nanoparticles have been explored and developed for similar photophysical and photochemical properties. Of particular interest and significance is the recent finding that small carbon nanoparticles could be surface-passivated by organic molecules or polymers to become highly photoactive, exhibiting strong photoluminescence in the visible and near-infrared spectral regions.\textsuperscript{9-15} These photoluminescent carbon nanoparticles, dubbed “carbon dots” (Scheme 2.1), were found to be physico-chemically and photochemically stable and non-blinking in the luminescent emissions.\textsuperscript{9} Here we report that the photoluminescence from carbon dots could be quenched highly efficiently by either electron acceptor or electron donor molecules in solution, namely that the photoexcited carbon dots are excellent as both electron donors and electron acceptors. These interesting photoinduced electron transfer properties may offer new opportunities in potentially using carbon dots for light energy conversion and
Scheme 2.1 Representation of a carbon dot containing an oligmeric PEG diamino surface passive agent
related applications, in addition to their being valuable to the effort on mechanistic elucidation.

2.2 Experimental Section

2.2.1 Materials

\(N,N\)-diethylaniline and diethylamine were purchased from Acros and purified by distillation. 4-nitrotoluene and 2,4-dinitrotoluene was purchased from Aldrich and purified by recrystallization in ethanol/H\(_2\)O (1:1) solution. Poly(ethylene glycol) diamine (\(\sim35\) repeating units for a molecular weight of 1,500, \(\text{PEG}_{1500N}\)) was from Fluka and dialysis membrane tubing from Spectrum Laboratories. Silver nitrate, Toluene, methanol and chloroform were supplied by VWR. Water was deionized and purified by being passed through a Labconco WaterPros water purification system.

2.2.2 Measurements

Beckman-Coulter ultracentrifuge (Optima L90K with a type 90 Ti fixed-angle rotor) were used. Transmission electron microscopy (TEM) imaging was carried out on Hitachi HD-2000 S-TEM system. Atomic force microscopy (AFM) images were obtained in the acoustic AC mode on Molecular Imaging PicoPlus system equipped with a multipurpose scanner and a NanoWorld Pointprobe NCH sensor. The height profile analysis was assisted by using the SPIP software distributed by Image Metrology. UV/vis absorption spectra were recorded on Shimadzu UV2101-PC spectrophotometer.
Photoluminescence spectra were measured on Spex Fluorolog-2 emission spectrometer equipped with a 450 W xenon source and a detector consisting of a Hamamatsu R928P photomultiplier tube operated at 950 V. For photo irradiation experiment, 450 W Xenon lamp was used for illumination, which is connect with a spectral monochromator (SPEX 1681). The irradiation wavelength is set at 450nm or 600 nm. The sample solution was put into a black sample chamber which is connected to the monochromator. Fluorescence decays were measured on a time-correlated single photon counting (TCSPC) setup with a Hamamatsu stabilized picosecond light pulser (PLP-02) for 407 nm excitation (<100 ps pulses at 1 MHz repetition rate), coupled with a Phillips XP2254/B PMT in a thermoelectrically cooled housing as detector for an overall instrument time resolution better than 500 ps.

### 2.2.3 Preparation of PEG<sub>1500N</sub>-attached Carbon Dots

Experimentally, carbon nanoparticles from laser ablation were refluxed in an aqueous nitric acid solution (2.6 M) for 12 h. After being cooled to room temperature, the sample was dialyzed against fresh water and dried by rot evaporation. The acid treated nanoparticles were refluxed in neat SOCl<sub>2</sub> for 6 h. After a complete removal of residual SOCl<sub>2</sub> on a rotary evaporator with a vacuum pump, the sample (100 mg) was mixed well with PEG<sub>1500N</sub> (1 g) in a flask, heated to 110 °C, and vigorously stirred under nitrogen protection for 3 days. It was then cooled to room temperature and dispersed in water, followed by centrifuging at 25,000g to retain the supernatant phase.
2.3 Results and Discussion

The carbon dots in this study were prepared by using the same procedures as those reported previously. In the preparation, the small carbon nanoparticles (separated from the laser ablation-produced powdery sample) were refluxed in aqueous nitric acid solution for the purpose of oxidizing surface carbons into carboxylic acids, followed by thionyl chloride treatment and then amidation with the oligomeric ethylene glycol diamine H\(_2\)NCH\(_2\)(C\(_2\)H\(_4\)O\(_{35}\))C\(_2\)H\(_4\)CH\(_2\)NH\(_2\) (PEG\(_{1500N}\)) to form the carbon dots with surface-attached PEGs (Scheme 2.1). The transmission electron microscopy (TEM) results (Figure 2.1) suggested that these dots were well-dispersed, with sizes averaging about 4.2 nm (based on statistical analyse of more than 300 dots), as also supported by the atomic force microscopy (AFM) results (Figure 2.1).

Photoluminescence spectra of the carbon dots in aqueous or organic solutions were generally broad (Figure 2.2) with luminescence emission intensities (425 nm excitation) which were quenched by the known electron acceptors 4-nitrotoluene (-1.19 V vs. NHE)\(^{16}\) and 2,4-dinitrotoluene (-0.9 V vs. NHE)\(^{17}\) in toluene solution, with the observed Stern–Volmer quenching constants (\(K_{SV} = \tau_0 k_q\)) from linear regression of 38 M\(^{-1}\) and 83 M\(^{-1}\), respectively (Figure 2.3). Obviously 2,4-dinitrotoluene was a much more effective quencher than 4-nitrotoluene, consistent with its being a significantly stronger electron acceptor. The luminescence decays of the carbon dots in the absence of
Figure 2.1 TEM (left) and AFM (right) images of the carbon dots used in this study. The TEM specimen was prepared by depositing a few drops of a diluted carbon dot solution onto a carbon-coated copper grid, followed by evaporation. The AFM specimen on a mica surface was similarly prepared.
Figure 2.2 Top: Luminescence emission spectra (425 nm excitation) of the carbon dots in toluene without (- - -) and with the indicated quenchers (both 0.016 M, —). Bottom: Luminescence decays (407 nm excitation, monitored with 470 nm narrow bandpass filter) of the carbon dots without (- - -) and with the quenchers (both 0.028 M, —).
quenchers could not be deconvoluted with a mono-exponential function (probably due to a distribution of emissive species and/or sites),\textsuperscript{18} but could be deconvoluted with the use of a multicomponent decay function to yield an average lifetime $\tau_{F}^{\circ}$ around 4 ns.\textsuperscript{9} Thus, on average the bimolecular rate constants $k_q$ for the quenching of luminescence emissions in the carbon dots by 4-nitrotoluene and 2,4-dinitrotoluene were on the order of $9.5 \times 10^9$ M$^{-1}$s$^{-1}$ and $2.1 \times 10^{10}$ M$^{-1}$s$^{-1}$, respectively. These, especially that for 2,4-dinitrotoluene, are beyond the upper limit for any bimolecular luminescence quenching processes in solution,\textsuperscript{18} highlighting the high efficiency of the underlying electron transfer and also suggesting the presence of static quenching contributions, which were confirmed by the Stern-Volmer plots from the observed average luminescence lifetimes (Figure 2.3). The corresponding quenching rate constants, $k_q$ of $\sim 6.5 \times 10^9$ M$^{-1}$s$^{-1}$ for 4-nitrotoluene and $8 \times 10^9$ M$^{-1}$s$^{-1}$ for 2,4-dinitrotoluene, are still at the diffusion-controlled limit for dynamic quenching.

The electron donating capabilities of the photoexcited carbon dots were also demonstrated in the photoreduction of Ag$^+$ to Ag. Experimentally, the reduction could be accomplished by photoirradiating (450 W xenon arc lamp coupled with a Spex 1681 monochromator) carbon dots in an aqueous solution of AgNO$_3$ at a visible wavelength such as 450 nm, which resulted in the emergence and rapid increases of the surface plasmon absorption owing to the increasing amount of Ag produced by the photoreduction. In order to avoid the subsequent irradiation into the surface plasmon absorption band of the initially formed Ag, the same experiment was also performed with
Figure 2.3 Stern-Volmer plots for the quenching of luminescence quantum yields (425 nm excitation) of the carbon dots by 2,4-dinitrotoluene (○) and 4-nitrotoluene (△) in toluene; and plots for the quenching of luminescence lifetimes (407 nm excitation) by 2,4-dinitrotoluene (●) and 4-nitrotoluene (▲). The lines represent the best fits (the least-square regression) of the respective data.
600 nm excitation, and similar photoreduction was observed. There was no Ag formation in control experiments in the absence of carbon dots, as expected.

Interestingly, the carbon dots were similarly strong electron acceptors as well, allowing highly efficient luminescence quenching by known electron donors such as \(N,N\)-diethylaniline (DEA, 0.88 V vs. NHE).\(^{19,20}\) As shown in Figure 2.4, the DEA quenching was also strongly solvent dependent, significantly more efficient in a polar solvent methanol than in chloroform. The Stern-Volmer plots for the quenching of luminescence quantum yields were curved downward at higher DEA concentrations, much more so for the quenching in methanol (Figure 2.4). The linear fits for only the data points at lower DEA concentrations yielded Stern-Volmer quenching constants \(K_{SV}\) of 19 M\(^{-1}\) and 5.1 M\(^{-1}\) in methanol and chloroform, respectively. The results from the quenching of luminescence lifetimes suggested no significant static quenching contributions. While not as extreme as those with electron acceptor quenchers discussed above, these Stern-Volmer constants are again corresponding to rate constants \(k_q\) toward the upper limit for bimolecular luminescence quenching processes in solution.\(^{18}\)

The strong solvent polarity dependence of the luminescence quenching by DEA is a good indication for an electron transfer quenching mechanism. As additional supporting evidence, the efficiency of the luminescence quenching was found to be strongly dependent on the electron donating ability of the quencher. For example, a weaker electron donor such as diethylamine (1.55 V vs. NHE)\(^{19}\) was considerably less efficient in the quenching of luminescence emissions in the carbon dots under otherwise the same
Figure 2.4 Stern-Volmer plots for the quenching of luminescence quantum yields (400 nm excitation) of the carbon dots by DEA in methanol (○, the line from fitting the data points up to 0.05 M) and chloroform (□, the line from fitting the data points up to 0.08 M), and for the quenching of luminescence lifetimes (407 nm excitation) in methanol (●). The low-concentration portion of the same plot for diethylamine as the quencher in methanol (—..—) is also shown for comparison.
experimental conditions (Stern-Volmer quenching constant $K_{SV}$ about $0.3 \text{ M}^{-1}$, Figure 2.4).

Mechanistically, the photoluminescence in carbon dots has been attributed to the energy trapping on the passivated carbon particle surface.$^{9-11}$ We speculate that there could even be phenomenological similarities between the luminescence emission mechanisms in traditional semiconductor quantum dots$^{1,2}$ and carbon dots (despite carbon being hardly a member of the semiconductor family), such that the emissions in carbon dots might also be a result of radiative recombination of surface-trapped electrons and holes. It is known that the carbon core in carbon dots must necessarily be very small (sub-10 nm or preferably sub-5 nm),$^{9-11}$ which should create inhomogeneous particle surface sites. Upon passivation via organic or polymeric functionalization, these surface sites could facilitate the trapping of photoinduced electrons and holes. As for the observed highly efficient quenching of luminescence emissions in the carbon dots by both electron acceptor and electron donor molecules,$^{21}$ their disruption to the radiative recombinations on the passivated carbon surface might be responsible. Further investigations including potentially probing directly the electron-hole pairs and/or their recombination processes in the photoexcited carbon dots are desired and should be pursued. Nevertheless, the substantial photoinduced redox properties of carbon dots reported here will open up new opportunities for these newly found quantum dots-like nanomaterials in light-harvesting and related applications.

(Chapter 2 has been published in the literature as reference 24.)
References


21. The fluorescence quenching of semiconductor QDs (CdSe and CdSe/ZnS, for example) by both electron donors and acceptors has also been reported. 6-8, 22, 23


CHAPTER THREE

BANDGAP-LIKE STRONG FLUORESCENCE IN FUNCTIONALIZED CARBON NANOPARTICLES

3.1 Introduction

Semiconductor quantum dots (QDs), especially the highly fluorescent CdSe-based core-shell nanostructures, have generated much excitement for their variety of potential applications in optical bioimaging and beyond.\textsuperscript{1,2} These QDs are widely considered as being more advantageous than conventional organic dyes and genetically engineered fluorescent proteins in terms of optical brightness and photostability.\textsuperscript{1,3-5} However, a serious disadvantage with these popular QDs is that they contain heavy metals, such as cadmium, whose significant toxicity and environmental hazard are well-documented.\textsuperscript{6-9} Therefore, alternative benign (nontoxic) QD-like fluorescent nanomaterials have been pursued, including the recent finding of fluorescent carbon nanoparticles (dubbed “carbon dots”).\textsuperscript{10,11}

Carbon dots are surface-passivated small carbon nanoparticles and the surface passivation is most effective following functionalization with organic or biomolecules\textsuperscript{10-16} (though other passivation schemes are also possible for weaker emissions\textsuperscript{17-19}). In addition to sharing some of the major advantageous characteristics of semiconductor QDs, including high photostability,\textsuperscript{1,10,13} large two-photon excitation cross-sections,\textsuperscript{11,20} and their applicability as optical imaging agents \textit{in vivo},\textsuperscript{20,21} carbon dots are also nonblinking,\textsuperscript{10,13} readily water soluble,\textsuperscript{10,11,13-16} and nontoxic according to currently available cytotoxicity and \textit{in vivo} toxicity evaluation results.\textsuperscript{18,22} The as-produced carbon
dots have so far exhibited fluorescence quantum yields of up to 20% in the green region of the spectrum,\textsuperscript{22} which are somewhat lower than those of the best-performing commercially available CdSe/ZnS QDs for the comparable spectral region.

Herein, we report that the as-prepared carbon dots sample could be fractionated simply on an aqueous gel column and the most fluorescent fractions achieved emission yields close to 60%, comparable to those of the best commercial CdSe/ZnS QDs in solution and brighter at the individual dot level (owing to the carbon dots being significantly higher in absorptivities). Interestingly, both the absorption and fluorescence results of the carbon dots resembled those of band-gap transitions, typically found in nanoscale semiconductors. The prospect of carbon particles on the nanoscale acquiring essentially semiconductor-like properties that are enhanced by surface functionalization is discussed.

3.2 Experimental Section

3.2.1 Materials

The diamine-terminated oligomeric poly(ethylene glycol) or PEG\textsubscript{1500N}, H\textsubscript{2}NCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}(OCH\textsubscript{2}CH\textsubscript{2})\textsubscript{n}CH\textsubscript{2}NH\textsubscript{2} (n \sim 35), and thionyl chloride were supplied by Aldrich. Sephadex G-100\textsuperscript{TM} gel was provided by GE Healthcare. Invitrogen aqueous compatible Qdot 525 ITKTM amino (PEG) CdSe/ZnS QDs sample (commonly referred to as “QD525PEG” in the literature) was purchased from the company. Water was
deionized and purified by being passed through a Labconco WaterPros water purification system.

3.2.2 Measurements

UV/vis absorption spectra were recorded on a Shimadzu UV2101-PC spectrophotometer. Fluorescence spectra were obtained on a Spex Fluorolog-2 emission spectrometer equipped with a 450 W xenon source and a detector consisting of a Hamamatsu R928P photomultiplier tube (PMT) operated at 950 V. Fluorescence decays were measured on a time-correlated single photon counting (TCSPC) setup with a Hamamatsu stabilized picosecond light pulser (PLP-02) for 407 nm excitation (<100 ps pulses at 1 MHz repetition rate), coupled with a Phillips XP2254/B PMT in a thermoelectrically cooled housing as detector for an overall instrument time resolution better than 500 ps.

Fluorescence quantum yields were measured by using quinine sulfate in 0.1 M H₂SO₄ solution (Φᵢ = 0.54) and 9,10-bis(phenylethynyl)-anthracene in cyclohexane (Φᵢ = 1.0) as fluorescence standards. The absorbance (optical density < 0.1 to minimize inner-filter effects) at the excitation wavelength was matched between the sample and the standard. The observed fluorescence spectra were corrected for nonlinear instrument response before the integration of their total intensities for the calculation of fluorescence quantum yields.

Transmission electron microscopy (TEM) imaging was performed on a Hitachi HD-2000 scanning TEM system in both transmission and Z-contrast modes. Atomic
force microscopy (AFM) images were obtained in the acoustic AC mode on a Molecular Imaging PicoPlus AFM system equipped with a multipurpose scanner and a NanoWorld Pointprobe NCH sensor. The height profile analysis was assisted by using the SPIP software distributed by Image Metrology.

Fluorescence imaging was carried out on a Leica laser scanning confocal fluorescence microscope (DM IRE2, with Leica TCS SP2 SE scanning system) equipped with an argon ion laser (JDS Uniphase). The specimens were prepared by vigorously diluting each sample solution and then dropping the solution onto a glass slide, followed by drying in ambient. The same instrumental conditions were carefully maintained when different specimens were compared. The fluorescence images were processed and analyzed by using the NIH ImageJ software.

3.2.3 Preparation of Carbon Dots

The preparation of precursor carbon nanoparticles and the synthesis of carbon dots were based on the previously reported procedures, with slight modifications and more rigorous controls of the experimental conditions for improved fluorescence properties. Briefly, the carbon soot was refluxed in aqueous nitric acid solution (2.6 M) for 12 h, dialyzed against fresh water, and then centrifuged at 1,000g to retain the supernatant. The recovered sample was refluxed in neat thionyl chloride for 6 h, followed by the removal of excess thionyl chloride on a rotovap. The treated carbon particle sample (100 mg) was mixed well with carefully dried PEG_{1500N} (1 g) in a flask, heated to 110 °C, and vigorously stirred under nitrogen protection for 3 days. The reaction mixture
was cooled to room temperature, dispersed in water, and then centrifuged at 25,000g to retain the supernatant.

3.2.4 Fractionation of Carbon Dots

The gel column for the fractionation of carbon dots was prepared with the commercially supplied Sephadex G-100\textsuperscript{TM} gel.\textsuperscript{23} Briefly, the gel (15 g) was soaked in water for 3 days, and the supernatant (including the suspended ultrafine gel) was discarded. The remaining gel was washed until no gel was suspended in the supernatant. Air bubbles were removed with vacuum. Separately, a glass column (25 mm inner diameter) was filled with water to remove air bubbles, and then closed. The gel suspension described above was poured into the column. As the gel precipitation to reach about 2 cm in height, the column was opened for the continuous addition of the gel suspension. The gel-filled column was washed until no changes in height (36 cm), followed by the testing and calibration of the column.\textsuperscript{23} In the fractionation, an aqueous solution of the as-prepared carbon dots was added to the gel column and eluted with water. Colored fractions were collected for characterization and further investigations.

3.3 Results and Discussion

The synthesis of carbon dots with an oligomeric PEG diamine (PEG\textsubscript{1500N}) as the surface passivation agent (Scheme 3.1) was based largely on the previously reported
Scheme 3.1 Representation of a carbon dot containing an oligomeric PEG diamino surface passive agent.
procedure,\textsuperscript{10,22} except for a more rigorous control of the functionalization reaction conditions (critical to the enhanced fluorescence performance in the resulting carbon dots). The precursor carbon nanoparticles were treated with thionyl chloride to generate acyl chlorides on the particle surface and then reacted in the melt of PEG\textsubscript{1500N} at 110 °C, for which the reaction temperature was found to significantly influence the fluorescence yield of carbon dots. The sample of carbon dots was processed in aqueous solution, and the resulting colored aqueous solutions at various concentrations remained stable indefinitely. The blue optical absorption shoulder (around 450 nm, Figure 3.1) was characteristic of these sample solutions, whilst the excitation resulted in equally characteristic green fluorescence emissions (centered around 510 nm, Figure 3.1) with quantum yields $\Phi_F$ of 16-20% (representing variations from batch to batch).

The as-prepared sample of carbon dots was loaded onto an aqueous gel column packed with Sephadex G-100 (supplied by GE Healthcare)\textsuperscript{23} for fractionation. With water as eluent, the fractions were collected and their optical absorption spectra were measured. As in the pre-fractionation sample, later fractions featured an increasingly well-defined absorption shoulder in the blue region (in the first fraction, the shoulder, which had a relatively lower intensity, was masked by other broad absorptions; Figure 3.1), and the excitation resulted in strong green fluorescence emissions. Whilst the observed fluorescence spectra were all rather similar (Figure 3.1), their quantum yields were significantly different, becoming progressively higher in the later fractions, and reaching $\Phi_F$ of 55-60% in the most fluorescent last fraction (Figure 3.2).
Figure 3.1 Absorption and fluorescence (440 nm excitation) spectra of the fractions 1 (a), 3 (b), 5 (c), and the most fluorescent 7 (d). Dashed lines in (d) represent the spectra of the “as-prepared” sample for comparison.
Figure 3.2 Fluorescence quantum yields (◯) and lifetimes (▲) of the different fractions, and the linear relationship between the observed yields and lifetimes (inset).
For comparative analyses on the nanoscale, the prefractionation sample and the most fluorescent fraction were deposited onto substrates for imaging using transmission electron microscopy (TEM) and atomic force microscopy (AFM). The TEM images suggested no major differences between the two samples under comparison, except for the latter sample containing on average slightly smaller particles, and a narrower distribution according to statistical analyses (Figure 3.3). These conclusions were generally supported by the AFM imaging results and the associated height analyses (Figure 3.4).

The fluorescence decay in the fractions could only be deconvoluted with a multiexponential function,\textsuperscript{24} to give an average fluorescence lifetime for each of the fractions. The variation in the lifetime values was consistent with that in the observed fluorescence quantum yields from different fractions (Figure 3.2), thus suggesting a relatively uniform fluorescence radiative process throughout the fractions (namely, that the observed fluorescence quantum yield variations were due predominantly to changes in the competing nonradiative processes from fraction to fraction). The fluorescence radiative rate constants \( k_F = \Phi_F / \tau_F \) were very large throughout the fractions, on average \( 1 \times 10^8 \text{s}^{-1} \), which suggests very strong electronic transitions.\textsuperscript{25,26} For reference, anthracene as a strongly fluorescent organic dye has a radiative rate constant \( k_F \) of less than \( 5 \times 10^7 \text{s}^{-1} \), to which the corresponding molar absorptivity of the 0–0 transition is more than 8000m\(^{-1}\)cm\(^{-1}\).\textsuperscript{26} Also, for comparison, the commercially supplied best-performing CdSe/ZnS QDs (“QD525PEG” from Invitrogen) were found to have a \( k_F \) value of
Figure 3.3 Representative TEM images of carbon dots in the as-produced sample (upper) and in the most fluorescent fraction (lower, and also the attached high-resolution images of two dots), with the corresponding statistical size analysis results based on multiple images.
Figure 3.4 AFM topography images of carbon dots in the most fluorescent fraction.
approximately $0.3 \times 10^8 \text{ s}^{-1}$ for the similar spectral region ($\Phi_F \approx 0.6$ and $\tau_F \approx 18.5 \text{ ns}$; determined experimentally under the same conditions).

According to well-established photophysical principles, the radiative rate constant is proportional to the integrated molar absorptivities in a particular absorption band, and in the first approximation proportional to the molar absorptivity at the band maximum. Therefore, ratio of the absorbance at the band maximum ($A_{\text{max}}$) to $k_F$ is approximately proportional to the numbers of dots in the solution; i.e., in a comparison between solutions of carbon dots and QDs, the same $A_{\text{max}}/k_F$ value essentially represents the same number of dots in both solutions. Such a comparison, shown in Figure 3.5, suggests that at the individual dot level the carbon dots in the most fluorescent fraction could fluoresce more than twice as brightly as the reference CdSe/ZnS QDs in the same spectral region. This supposition was supported by results from the single-dot fluorescence imaging experiments described below.

The carbon dots were dispersed on cover glass used as a substrate in infinite dilution to allow confocal microscopy imaging of individual dots. The deposition conditions for the preparation of the specimens were essentially the same as those for TEM and AFM imaging, and the results confirmed the dispersion of individual dots in the specimens. For the prefractionation sample, fluorescence images of carbon dots that had a wider range of brightness were observed (Figure 3.6), which was consistent with the fact that the sample contained fractions of different fluorescence quantum yields. As expected, the carbon dots in the specimen from the most fluorescent fraction were more uniform in terms of fluorescence brightness (Figure 3.6). Also as expected from the
Figure 3.5 Absorption (ABS) and fluorescence (FLSC) spectra of carbon dots in the most fluorescent fraction (---) are compared with those of Invitrogen QD525PEG QDs (—) in aqueous solutions (upper, FLSC intensities corresponding to excitations at matching first band maximum $A/k_F$ values), and with those of ZnS-doped carbon dots$^{34}$ (lower).
Figure 3.6 Fluorescence microscopy images (458 nm excitation) of carbon dots in as-prepared sample (upper left) and in the most fluorescent fraction (lower left), and images of Invitrogen QD525PEG QDs (upper right). The bar-chart comparison was based on averaging 300 most fluorescent dots in each of the three samples.
conclusion in the comparison between bulk solutions of the same $A_{\text{max}}/k_F$ ratio (discussed above), the individual carbon dots in this fraction had a noticeably brighter fluorescence (mostly by 2-2.5 fold; Figure 3.6) than the CdSe/ZnS QDs.

The carbon dots are comparable in size with, or somewhat smaller than, the commercially available aqueous-compatible CdSe/ZnS QDs (especially when the surface-capping agents are included in the dot sizes). Therefore, the brighter fluorescence emissions in individual carbon dots make these dots particularly valuable for optical bioimaging in vitro and in vivo, especially with regard to the emerging needs for molecular probes in high-resolution cellular imaging.\textsuperscript{27,28}

Mechanistically, the fluorescence in carbon dots was thought to be associated with passivated surface defects of the core carbon particles.\textsuperscript{10,11} In previous reports on the trapping of excited-state energy by surface defects in the nanoparticles, the emissive states were generally different from the initially excited state.\textsuperscript{29,30} For nanoscale semiconductors such as CdS, as a classical example, the excitation into the band-gap absorption band resulted in exciton fluorescence and, in most cases, surface-defect emissions.\textsuperscript{29–32} These surface-defect emissions may even be overwhelming in the observed fluorescence spectra of many CdS nanoparticles.\textsuperscript{30,33} In carbon dots, on the other hand, there are no classical band-gap absorptions, so the surface-defect states must be accessed directly from the ground state. Therefore, the trapping of excited-state energy probably occurs between the defects responsible for absorptions and those for emissions (instead of between the excitonic state and the emissive defect states found in CdS and other semiconductor nanoparticles). One may thus expect a broad distribution of
excitations, corresponding to mostly featureless absorption spectra, as are typically observed for carbon dots.\textsuperscript{10,13,14} Interestingly and importantly, however, the spectroscopic results reported here suggest that the electronic transitions in carbon dots are not necessarily broadly distributed.

The absorption shoulder in the blue-light region (Figure 3.1) is in fact surprisingly well-defined and specific in all of the more-fluorescent later fractions and in the prefractionation sample as well, in which the more rigorously controlled reaction conditions in the synthesis of carbon dots apparently enhanced the absorption shoulder at the expense of broad absorptions at other colors. The same absorption feature was also observed previously in the “doped” carbon dots (Figure 3.5), in which the carbon core was doped with an insoluble inorganic salt, such as ZnO or ZnS.\textsuperscript{34} Of particular interest is that the ZnO or ZnS doping also resulted in substantially more-fluorescent carbon dots,\textsuperscript{34} rather similar to the fractionated carbon dots obtained previously in terms of both optical absorption and fluorescence properties (Figure 3.5). It seems that the absorption shoulder around 450 nm and the corresponding fluorescence band around 510 nm represent “sweet spots” in the electronic transitions, because they are apparently shared by the carbon dots of different surface functionalities. These preferred transitions in the carbon dots are almost as specific as the band-gap transitions that are characteristic of quantum-confined nanoscale semiconductors. Phenomenologically at least, nanoscale carbon particles that have the appropriate surface functionalization (as in the later fractions reported here) or other forms of surface passivation, such as a combination of doping with inorganic salt and organic functionalization,\textsuperscript{34} could become semiconductor-like to exhibit band-gaplike
electronic transitions. In terms of optical properties at least, the surface passivated small carbon nanoparticles seem no different from quantum-confined semiconductors.

An interesting question with potentially far-reaching implications is whether such specific electronic transitions in the carbon dots in this work could be found or even tuned to other colors. At present, we have insufficient experimental data available to provide an affirmative answer to this question, although the broad absorption and fluorescence spectra (covering the entire visible spectral region and extending into the near-IR region) observed in the preparations of other carbon dots do suggest that carbon dots are, at least in principle, capable of direct electronic transitions at many other wavelengths.

The changes in fluorescence quantum yield and lifetime among the different fractions might be explained by varying the degree of surface passivation by PEG$_{1500N}$ molecules, both covalently through amide linkages and noncovalently through strong surface adsorption, and an influence from the differences in particle size. Because the free PEG$_{1500N}$ molecules eluted slowest from the gel column, we expect that the later fractions probably consisted of carbon dots that were somewhat smaller in size and well passivated with PEG$_{1500N}$ molecules (thus making the dots behave more similarly to free PEG$_{1500N}$ molecules). However, we have not yet obtained the quantitative results required to confirm or disprove this theory, as structural elucidation of the carbon dots using NMR and FTIR analysis has been rather difficult. For example, $^{13}$C NMR spectra were generally simple but not informative, exhibiting only the expected weak carbonyl signals.
(other particle surface carbons were not detected owing to their being too diverse). Further investigations are necessary and will be pursued.

Even without a clear structural understanding of the carbon dots in the most fluorescent fraction, the existence of these dots itself is very important fundamentally and mechanistically, and the successful isolation of these brightly fluorescent carbon dots reported here may be highly valuable technologically. The fact that these carbon dots are individually much brighter than their comparable semiconductor QDs, coupled with their nontoxicity (at least on the basis of presently available results),\textsuperscript{18,22} should lead to significant applications in bioimaging and beyond.

(Chapter 3 has been published in the literature as reference 35.)
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4.1 Doped Carbon Nanoparticles as a New Platform for Highly Photoluminescent Dots

4.1.1 Introduction

There have been rapid advances in the development and applications of semiconductor quantum dots (QDs), especially for the more fluorescent core-shell dots based on CdSe nanocrystals with a wide-bandgap semiconductor shell. Despite their demonstrated performance and widely discussed potentials, however, a major limitation is their necessary use of heavy metals such as cadmium. In the continuing search for benign (nontoxic) alternatives, Sun and co-workers found and reported that nanosized pure carbon particles may be surface-passivated by organic molecules (dubbed “carbon dots”) to exhibit bright photoluminescence in the visible with either one- or two-photon excitation. Carbon dots compare favorably with the semiconductor QDs in many properties (carbon being a nontoxic element, no-blinking, etc.), but their brightness (emission quantum yields up to 15-20%) is still lower than that of the best-performing CdSe/ZnS core-shell dots. In this work, we found that carbon nanoparticles may be doped with inorganic salts such as ZnO or ZnS before their surface passivation by organic molecules to achieve much higher photoluminescence quantum yields. These new dots with a doped carbon core (Figure 4.1.1) are performance-wise competitive to the commercially available CdSe/ZnS dots, especially in aqueous solutions (where
Figure 4.1.1 Left: cartoon illustration on carbon dots with a doped carbon core (from an experimental HR-TEM image with ZnS lattice fringes circled). Right: aqueous solutions of C\textsubscript{ZnS}-Dots and C\textsubscript{ZnO}-Dots (450 nm excitation for both) compared with a commercial toluene solution of CdSe/ZnS dots (matching optical density at excitation), all photographed through a 475 nm cutoff filter.
shortcomings of the CdSe/ZnS dots are known in the literature \(^3b,^{11}\). The results suggest that small salt-doped carbon nanoparticles represent a new platform for quantum dotlike optical nanomaterials.

4.1.2 Experimental Section

4.1.2.1 Materials

Zinc acetate dihydrate and sodium sulfide were purchased from Alfa, sodium hydroxide from Aldrich, and the poly(ethylene glycol) diamine (\(\sim 35\) repeating units for a molecular weight of 1500) from Fluka. \(N, N\)-Dimethylformamide and sodium dodecyl sulfate were supplied by Acros and VWR, respectively. Millipore Durapore membrane filters (0.22 \(\mu\)m, GV membrane) were obtained from Fisher Scientific, dialysis membrane tubing from Spectrum Laboratories, and carbon- and silicon-coated copper grids from Electron Microscopy Sciences. Water was deionized and purified by being passed through a Labconco WaterPros water purification system.

For the ZnS doping, the laser ablation-produced carbon nanoparticle sample\(^8,^9\) (1 g) was refluxed in an aqueous nitric acid solution (2.6 M) for 12 h, neutralized via dialysis (membrane molecular weight cutoff \(\sim 1000\)) against a large volume of fresh water, and then centrifuged at 1000g for 5 min. The supernatant was retained and evaporated to remove water. The recovered carbon nanoparticles (600 mg) were dispersed in DMF (200 mL) with the aid of ultrasonication (VWR model 250D) for 30 min. To the suspension was added zinc acetate dihydrate (680 mg, 3.1 mmol) under vigorous stirring, followed by slow dropwise addition of an aqueous Na\(_2\)S solution (0.62
M, 5 mL) at room temperature. The mixture was centrifuged at 3000g, and the precipitate was retained and repeatedly washed with distilled water to obtain the ZnS-doped carbon nanoparticles (881 mg).

In the doping with ZnO, the same initial treatments of carbon nanoparticles were applied to obtain their dispersion in DMF (600 mg/200 mL). To the dispersion was added zinc acetate dihydrate (680 mg, 3.1 mmol) under vigorous stirring, followed by slow dropwise addition of an aqueous NaOH solution (1.25 M, 5 mL) at room temperature. The mixture was centrifuged at 3000g to discard the supernatant. The precipitate was repeatedly washed with water, evaporated to remove water, and then dried at 60 °C in a vacuum oven. The sample was annealed at 200 °C for 2 h to obtain the ZnO-doped carbon nanoparticles (830 mg).

4.1.2.2 Measurements

Baxter Megafuge (model 2630) and Beckman-Coulter ultracentrifuge (Optima L90K with a type 90 Ti fixed-angle rotor) were used for low- and high-speed centrifugations, respectively. Thermogravimetric analysis (TGA) was performed on a TA Instruments Q500 TGA (up to 800 °C with air or nitrogen gas).

Transmission electron microscopy (TEM) imaging was carried out on a Hitachi HD-2000 S-TEM system and a Hitachi H-9500 TEM system. The same S-TEM system was used for the in situ energy dispersive X-ray spectroscopy (EDX) analysis. Atomic force microscopy (AFM) images were obtained in the acoustic AC mode on a Molecular Imaging PicoPlus system equipped with a multipurpose scanner and a NanoWorld
Pointprobe NCH sensor. The height profile analysis was assisted by using the SPIP software distributed by Image Metrology.

UV/vis absorption spectra were recorded on a Shimadzu UV2101-PC spectrophotometer. Photoluminescence spectra were measured on a Spex Fluorolog-2 emission spectrometer equipped with a 450 W xenon source and a detector consisting of a Hamamatsu R928P photomultiplier tube operated at 950 V. A Leica laser scanning confocal fluorescence microscope (DMIRE2, with Leica TCS SP2 SE scanning system) was used for optical imaging and spectral measurements. The microscope was equipped with an argon ion laser (JDS Uniphase) and a femtosecond pulsed (∼100 fs at 80 MHz) Ti:Sapphire laser (Spectra-Physics Tsunami with a 5 W Millennia pump). An oil immersion objective lens (Leica X63/1.40) was used in both one- and two-photon imaging experiments. For the two-photon measurements, an external nondescanned detector (NDD) was used for higher signals.

4.1.3 Results and Discussion

Experimentally, carbon nanoparticles from laser ablation were processed in terms of the nitric acid treatment, dialysis, and then centrifugation to retain the supernatant, in which the suspended nanoparticles were generally less than 10 nm in size according to electron microscopy analyses. The doping of the carbon nanoparticles with ZnO or ZnS was achieved in an aqueous suspension of the nanoparticles with Zn(CH$_3$COO)$_2$ through hydrolysis with NaOH or precipitation with Na$_2$S, respectively. For the former, the
sample was thermally annealed to convert Zn(OH)$_2$ to ZnO. No thermal annealing step was necessary for ZnS-doped carbon nanoparticles.

A sample (200 mg) containing either ZnO- or ZnS-doped carbon nanoparticles was dispersed in an aqueous solution of sodium dodecyl sulfate (1 wt %, 120 mL) via sonication for 30 min. Upon filtration, the filter cake was washed repeatedly with water, dried, and then mixed thoroughly with the diaminepolyethylene glycol H$_2$NCH$_2$(CH$_2$CH$_2$O)$_{35}$CH$_2$CH$_2$CH$_2$NH$_2$ (PEG$_{1500N}$, 1.9 g). The mixture was heated to 110 °C and stirred for 72 h under nitrogen protection. It was then cooled to room temperature and dispersed in water, followed by centrifuging at 25000 g to retain the supernatant. The reaction conditions were the same as those used previously in the functionalization of carbon nanotubes,$^{12}$ where the PEG$_{1500N}$ amino groups and the carboxylic acid moieties on the oxidized carbon surface (still naked areas on ZnO- or ZnS-doped carbon nanoparticles) form zwitterion pairs.$^{12,13}$ Additionally, there may also be strong PEG$_{1500N}$ adsorption on the particle surface, as also observed in the functionalized carbon nanotubes.

The carbon dots with ZnO- or ZnS-doped carbon cores ("C$_{ZnO}$-Dots" or "C$_{ZnS}$-Dots", respectively) were characterized by using microscopy techniques. Shown in Figure 4.1.2 are the TEM images of C$_{ZnS}$-Dots, which suggest typical dot sizes around 4-5 nm. At a higher imaging resolution, the doping of a carbon particle with ZnS could be visualized (Figure 4.1.2 and also Figure 4.1.1). The results from energy dispersive X-ray (EDX) analyses of C$_{ZnS}$-Dots on a silicon grid confirmed the presence of C, Zn, and S. Also shown in Figure 4.1.2 are AFM images of C$_{ZnO}$-Dots on a mica substrate.
Figure 4.1.2 (a) TEM (Z-contrast) images of $C_{\text{ZnS}}$-Dots. (b) High-resolution TEM images of individual carbon dots without doping (left) and with ZnS-doping (right, showing lattice fringes). (c) AFM topography images of $C_{\text{ZnO}}$-Dots on a mica substrate (and the height profile along the line).
Thermogravimetric analysis (TGA) measurements of the C\textsubscript{ZnO}-Dots and C\textsubscript{ZnS}-Dots samples were performed at 10 °C/min, first to 600 °C in nitrogen to remove the surface functional groups and then to 800 °C in air to oxidize the carbon core into carbon dioxide (purged out of the system). According to the TGA results, the core C:ZnO and C:ZnS ratios in C\textsubscript{ZnO}-Dots and C\textsubscript{ZnS}-Dots were approximately 3:1 and 2:1, respectively, in terms of weight (corresponding to 20:1 and 13:1, respectively, in molar ratios).

The absorption and luminescence emission spectra of C\textsubscript{ZnO}-Dots and C\textsubscript{ZnS}-Dots are rather similar (Figure 4.1.3). For both samples, the absorption spectra feature a shoulder in the blue region, where the absorptivities are on the order of 100 [(mole of core carbon atoms)/L]^{-1}cm^{-1}. The excitation into the absorption shoulder results in strong bluish green luminescence emissions (Figure 4.1.3 and also Figure 4.1.1). The observed emission quantum yields (440 nm excitation, quinine sulfate as the fluorescence standard) for C\textsubscript{ZnS}-Dots in aqueous solution are consistently higher than 50% (varying somewhat from batch to batch, up to 15%, in about a dozen of repeated sample preparations). This is competitive to the performance of commercially available organic-based CdSe/ZnS core-shell dots (NN-LABS, LLC, Figure 4.1.1).

The currently available C\textsubscript{ZnO}-Dots in aqueous solution are slightly less luminescent than C\textsubscript{ZnS}-Dots, with observed quantum yields around 45% (also varying somewhat from batch to batch, up to 15%, in repeated sample preparations).

Mechanistically, the photoluminescence in carbon dots has been attributed to passivated defects on the carbon particle surface acting as excitation energy traps, for which the covalently attached organic molecules serve as the passivation agents.\textsuperscript{8,9} While
Figure 4.1.3 Absorption (ABS) and luminescence emission (FLSC, 440 nm excitation, normalized against the peak intensity) spectra of C_{ZnS}-Dots (left) and C_{ZnO}-Dots (right) in aqueous solutions. As also shown for comparison, the carbon nanoparticles doped with ZnS or ZnO but without PEGs were not emissive (dashed lines, ×10 and offset by 0.1 for easier viewing).
the role of ZnO or ZnS doping in the substantial enhancement of photoluminescence performance is not clear (no precedent to follow), we propose that the dopant may provide secondary yet more effective surface passivation in combination with the organic passivation agents. Results from repeated control experiments suggested that the functionalization of ZnO- or ZnS-doped carbon nanoparticles by the organic (PEG\textsubscript{1500N}) molecules is necessary for the observed very strong photoluminescence, as compared in Figure 4.1.3.

The C\textsubscript{ZnO}-Dots and C\textsubscript{ZnS}-Dots are both strongly luminescent under multiphoton excitation conditions, a property that they share with the original carbon dots.\textsuperscript{9} The two-photon excitation at 800 nm with a femtosecond pulsed laser (Spectra Physics Tsunami Ti-Sapphire) resulted in bright luminescence emissions in the visible region, which were generally similar to those observed with one-photon excitation at 458 nm (argon ion laser). For example, shown in Figure 4.1.4 are luminescence images of C\textsubscript{ZnS}-Dots obtained on a confocal microscope (Leica DMIRE2 with TCS SP2 SE scanning system) with one- and two-photon excitations. Even with infinite dilution of the solution used in the preparation of the specimen, the resulting luminescence emissions from presumably individual dots could still be readily detected (Figure 4.1.4). These results suggest great potentials of these dots in one- and two-photon luminescence imaging applications.

In summary, small carbon nanoparticles doped with inorganic salts apparently serve as a highly promising new platform in the development of quantum dotlike optical nanomaterials for imaging and other applications. The C\textsubscript{ZnO}-Dots and C\textsubscript{ZnS}-Dots in aqueous solutions are competitive to the commercially available organic-based CdSe/ZnS
Figure 4.1.4 One- (left, 458 nm excitation) and two-photon (right, 800 nm excitation) luminescence images of the $\text{C}_{\text{ZnS}}$-Dots and that for the specimen from an infinitely diluted solution (left inset).
QDs in luminescence brightness. Beyond the blue-green regions, carbon dots with doped carbon cores for other colors are being pursued.

(Chapter 4.1 has been published in the literature as reference 14.)
References


4.2 Carbon Dots with the Core Doped by Different Inorganic Salts

4.2.1 Introduction

There has been extensive recent research in semiconductor quantum dots, especially CdSe/ZnS quantum dots, regarding their optical performance\textsuperscript{1,2} and related applications.\textsuperscript{3-8} However, because the toxicity of these substances greatly limits their potential applications,\textsuperscript{9-13} alternative nontoxic fluorescent nanoparticles,\textsuperscript{14-16} such as luminescent surface-passivated carbon nanoparticles, dubbed “carbon dots”, have also been a subject of intense research.\textsuperscript{17-20} The as-produced carbon dots exhibited fluorescence quantum yields up to 20%, which have further been improved to over than 50% through additional ZnS or ZnO doping.\textsuperscript{19} The fractionation of as-produced carbon dots by gel column yielded the most fluorescent fractions up to 60% in quantum yields, with an approximate brightness three times than that of the best commercial CdSe/ZnS QDs at the individual dot level (owing to the carbon dots being significantly higher in absorptivities).\textsuperscript{20} Toxicity evaluations showed that carbon dots are nontoxic both \textit{in vitro} and \textit{in vivo},\textsuperscript{21} indicating the greater suitability of carbon dots over CdSe/ZnS QDs for biological applications.\textsuperscript{21,22} In this section, in addition to ZnS and ZnO, we report that carbon dots can be doped by other inorganic salts such as TiO\textsubscript{2}, SiO\textsubscript{2}, AgCl and Fe\textsubscript{3}O\textsubscript{4}, and that the spectroscopic performances of carbon dots (especially fluorescence quantum yields) are strongly related to the types of dopants. Further fractionating doped carbon dots shows that the quantum yields of the most fluorescent fractions of ZnS-doped carbon dots could
reach up to 75%. Spectroscopic properties of carbon dots, which were changed by various dopants and the gel-column fractionation, are also discussed.

4.2.2 Experimental Section

4.2.2.1 Materials

$O,O'$-bis(3-aminopropyl) polyethylene glycol ($M_w \sim 1,500$, PEG$_{1500N}$), thionyl chloride ($\text{SOCl}_2$, >99%), titanium ethoxide ($\text{Ti(OC}_2\text{H}_5)_4$, >97%) and tetraethyl orthosilicate ($\text{Si(OC}_2\text{H}_5)_4$, >99%) were supplied by Aldrich. Zinc acetate dihydrate ($\text{Zn(OOCCH}_3\text{)}_2\cdot\text{2H}_2\text{O}$, >98%), sodium sulfide ($\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$, >98%), silver nitrate ($\text{AgNO}_3$, >99%), sodium chloride ($\text{NaCl}$, >99.5%), sodium hydroxide ($\text{NaOH}$, >97%), iron(II) sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, >98%) and iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, >98%) were purchased from Alfa. Nitric acid ($\text{HNO}_3$, 60-70%), ethanol ($\text{EtOH}$, >99%), 2-propanol ($((\text{CH}_3)_2\text{CHOH}$, 99.9%), $N,N$-dimethylformamide (DMF, 99%) and sodium dodecyl sulfate (SDS, 99%) were supplied by VWR. Sephadex G-100™ gel was provided by GE Healthcare. Carbon-coated and silica-coated copper grids were obtained from Electron Microscopy Sciences. Millipore Durapore membrane filters (pore size 0.22 µm) were purchased from Fisher. Water was deionized and purified using a Labconco WaterPros water purification system.
4.2.2.2 Measurements

The VWR model 250D bath sonicator and the Baxter megafuge model 2630 (up to 6000g) and Beckman Coulter model Optima L-90K (up to 694,000g) centrifuges were used. Thermogravimetric analysis was performed using a TA Instruments TGA Q500 analyzer, and X-ray powder diffraction measurements were performed on a Scintag XDS-2000 powder diffraction system. Transmission electron microscopy (TEM) imaging was conducted on a Hitachi H-9500 TEM system and a Hitachi HD-2000 S-TEM system, respectively with the latter also being used for the energy dispersive X-ray spectroscopy (EDX) analysis. Atomic force microscopy (AFM) images were obtained in the acoustic AC mode on a Molecular Imaging PicoPlus AFM system equipped with a multipurpose scanner and a NanoWorld Pointprobe NCH sensor. The height profile analysis was determined by using the SPIP software distributed by Image Metrology, and UV/vis absorption spectra were recorded on a Shimadzu UV2101-PC spectrophotometer. Fluorescence spectra were obtained on a Spex Fluorolog-2 emission spectrometer equipped with a 450 W xenon source and a detector consisting of a Hamamatsu R928P photomultiplier tube (PMT) operated at 950 V. Fluorescence decays were measured on a time-correlated single photon counting (TCSPC) setup with a Hamamatsu stabilized picosecond light pulser (PLP-02) for 407 nm excitation (<100 ps pulses at 1 MHz repetition rate), coupled with a Phillips XP2254/B PMT in a thermoelectrically cooled housing as detector for an overall instrument time resolution better than 500 ps.
4.2.2.3 Preparation of carbon dots and doped carbon dots

**Acid-treated carbon nanoparticles.**\(^{17}\) Carbon soot (2 g) obtained from laser ablation was refluxed in an aqueous nitric acid solution (2.6 M, 200 mL) for 12 h. After being cooled to room temperature, the sample was dialyzed against fresh water, followed by centrifuging at 1,000 g to retain the supernatant. Acid-treated carbon nanoparticles (1 g) were obtained after removal of the water.

**ZnS-doped carbon nanoparticles (C/ZnS).**\(^{19}\) Acid-treated carbon nanoparticles (600 mg) were dispersed in DMF (200 mL) with the aid of ultrasonication for 30 min. Zinc acetate dihydrate (680 mg, 3.1 mmol) was added to the suspension under vigorous stirring, followed by the slow dropwise addition of an aqueous Na\(_2\)S solution (0.62 M, 5 mL) at room temperature. The mixture was centrifuged at 3000 g, and the precipitate was retained and repeatedly washed with distilled water to obtain the ZnS-doped carbon nanoparticles (881 mg).

**ZnO-doped carbon nanoparticles (C/ZnO).** Acid-treated carbon nanoparticles (200 mg) was dispersed in 2-propanol (300 mL) through ultrasonication for 30 min. Zinc acetate dihydrate (180 mg, 0.82 mmol) was added to the suspension under vigorous stirring. The mixture was sonicated for 1 h and then cooled to 0 °C. A NaOH (53 mg, 1.31 mmol) in 2-propanol (67 mL) solution was then added at 0 °C within 1 min under stirring. It was then warmed to room temperature and then stirred continuously overnight. Upon removal of the solvent through rotovary evaporation, the residue was repeatedly washed with distilled water to obtain the ZnO-doped carbon nanoparticles (268 mg).
**TiO$_2$-doped carbon nanoparticles (C/TiO$_2$).** The sol-gel solution$^{23}$ was prepared as a doping source, with the molar ratio for the sol-gel solution preparation of Ti(OC$_2$H$_5$)$_4$:EtOH:H$_2$O:HNO$_3$ = 1:70:1.9:0.2. The solution was refluxed at 80 °C for 1 h under vigorous stirring. Acid-treated carbon nanoparticles (200 mg) were mixed with the solution (32 mL), and the mixture was sonicated for 1h, stirred for 12 h and then filtrated. The filter cake was grounded and annealed at 250 °C for 1 h to yield TiO$_2$-doped carbon nanoparticles (225 mg).

**SiO$_2$-doped carbon nanoparticles (C/SiO$_2$).** The sol-gel solution$^{23}$ was prepared as a doping source. The molar ratio for the sol-gel solution preparation was Si(OC$_2$H$_5$)$_4$:EtOH:H$_2$O:HNO$_3$ = 1:70:1.9:0.2. The solution was refluxed at 80 °C for 1 h under vigorous stirring. Acid-treated carbon nanoparticles (200 mg) were mixed with the solution (32 mL), and the mixture was sonicated for 1h, stirred for 12 h and then filtrated. The filter cake was grounded and annealed at 250 °C for 1 h to yield SiO$_2$-doped carbon nanoparticles (210 mg).

**AgCl-doped carbon nanoparticles (C/AgCl).** Acid-treated carbon nanoparticles (600 mg) were dispersed in DMF (200 mL) with the aid of ultrasonication for 30 min. Silver nitrate (357 mg, 2.1 mmol) was added to the dispersion under vigorous stirring, followed by a slow dropwise addition of an aqueous NaCl solution (0.42 M, 5 mL) at room temperature. The mixture was centrifuged at 3000g, and the precipitate was retained and repeatedly washed with distilled water to obtain the AgCl-doped carbon nanoparticles (876 mg).
**Fe₃O₄-doped carbon nanoparticles (C/Fe₃O₄).** Acid-treated carbon nanoparticles (200 mg) were dispersed in H₂O (200 mL) via ultrasonication for 30 min. Iron (III) chloride hexahydrate (232 mg, 0.86 mmol) and iron(II) sulfate heptahydrate (120 mg, 0.43 mmol) was added to the suspension under vigorous stirring at 80 °C in a nitrogen atmosphere, followed by slow dropwise addition of an aqueous NaOH solution (1.04 M, 5 mL) at room temperature. The mixture was centrifuged at 3000g, and the precipitate was retained and repeatedly washed with distilled water to obtain the Fe₃O₄-doped carbon nanoparticles (301 mg).

**Preparation of doped carbon dots**¹⁹ (Cₓ-Dots, X = ZnS, ZnO, TiO₂, SiO₂, AgCl, Fe₃O₄). Doped carbon nanoparticles (200 mg) were dispersed in an aqueous solution of sodium dodecyl sulfate (1 wt %, 120 mL) via sonication for 30 min. Upon filtration, the filter cake was washed repeatedly with water, dried, and then mixed thoroughly with carefully dried PEG₁₅₀₀₅ (1.9 g). The mixture was heated to 110 °C and stirred for 3 days under nitrogen protection. The mixture was then cooled to room temperature and dispersed in water, and then centrifuged at 25,000g to retain the supernatant as aqueous solution of the doped carbon dots.

**Fractionation of doped carbon dots.**²⁰ The gel column for the fractionation of doped carbon dots was prepared with the commercially supplied Sephadex G-100™ gel. Briefly, the gel (15 g) was soaked in water for 3 days, and the supernatant (including the suspended ultrafine gel) was discarded. The remaining gel was washed until no gel was suspended in the supernatant. Air bubbles were removed with vacuum. Separately, a glass column (25 mm inner diameter) was filled with water to remove air bubbles, and
then closed. The gel suspension described above was poured into the column. As the gel precipitation to reach about 2 cm in height, the column was opened for the continuous addition of the gel suspension. The gel-filled column was washed until no changes in height (36 cm), followed by the testing and calibration of the column. In the fractionation, an aqueous solution of C\textsubscript{ZnS}-Dots or C\textsubscript{TiO2}-Dots was added to the gel column and eluted with water. Colored fractions were collected for characterization and further investigation.

4.2.3 Results and discussions

We reported previously an investigation on the PEG\textsubscript{1500N} functionalized ZnS- or ZnO-doped carbon nanoparticles (C\textsubscript{ZnS}-Dots or C\textsubscript{ZnO}-Dots).\textsuperscript{19} The pre-functionalization ZnS- or ZnO-doped carbon nanoparticles (C/ZnS or C/ZnO) were synthesized using the following method. First, carbon nanoparticles were dispersed in DMF via the aid of ultrasonication. A layer of inorganic salts was then deposited onto the surface of carbon nanoparticles by sequential adsorption of metal cations onto the particle surface through electrostatic attraction and then slow reacted with non-metal anions to form insoluble salts. Specifically, ZnO in C/ZnO were synthesized through the reaction between Zn\textsuperscript{2+} and OH\textsuperscript{-} in an aqueous DMF solution. Since the formation of ZnO is not as favorable as the formation of Zn(OH)\textsubscript{2} in an aqueous environment,\textsuperscript{24} the obtained doped samples were annealed at 200 °C to convert Zn(OH)\textsubscript{2} to ZnO.\textsuperscript{19} In the work presented here, the procedure of preparing C/ZnO was modified by using anhydrous 2-propanol as the solvent to directly produce ZnO without thermal treatment.\textsuperscript{24,25} In this procedure, ZnO in C/ZnO was formed in 2-propanol according to the following reactions:
The C/ZnO obtained from the 2-propanol was functionalized in the classical thermal reaction with the PEG$_{1500N}^{17,19,21}$ to yield aqueous soluble C$_{ZnO}$-Dots. C/ZnS and C$_{ZnS}$-Dots were synthesized by using the same method described previously.$^{19}$ Both C$_{ZnS}$-Dots and C$_{ZnO}$-Dots were characterized by using microscopy techniques. The TEM images of C$_{ZnS}$-Dots (Figure 4.2.1) and C$_{ZnO}$-Dots (Figure 4.2.2) suggest that the typical particle sizes of both samples were approximately 5 nm. In high-resolution TEM images (Figure 4.2.1 and Figure 4.2.2), the carbon cores of C$_{ZnS}$-Dots or C$_{ZnO}$-Dots were partially coated by crystalline structures that can be assigned to either ZnS or ZnO. The results from energy dispersive X-ray (EDX) analyses confirmed the presence of elements C, Zn and S in C$_{ZnS}$-Dots (on a silicon grid), and C and Zn in C$_{ZnO}$-Dots (on a silicon grid).

Thermogravimetric analysis (TGA) and X-ray powder diffraction (XRD) were performed to measure both the pre- and post-functionalization doped carbon nanoparticle samples. The pre-functionalization doped carbon nanoparticle samples (C/ZnS or C/ZnO) were dried in a vacuum oven overnight prior to the measurement. The post-functionalization doped carbon nanoparticle samples (C/ZnS-post or C/ZnO-post), representing the doped carbon cores in the carbon dot samples, were obtained from the aqueous solution of C$_{ZnS}$-Dots or C$_{ZnO}$-Dots by being subsequently dried by rotovap and annealed at 600 °C for 30 min to selectively remove functional groups (PEG$_{1500N}$). TGA measurements were conducted with a relatively slow heating rate of 10 °C/min to 800 °C.
Figure 4.2.1 A representative TEM (Z-contrast) image of C\textsubscript{ZnS}-Dots (upper) and high-resolution TEM images of the individual C\textsubscript{ZnS}-Dots (lower), with corresponding statistical size analysis results based on multiple images (TEM images courtesy of Dr. Mohammed J. Meziani)
Figure 4.2.2 A representative TEM (Z-contrast) image of $\text{C}_\text{ZnO}$-Dots (left) and high-resolution TEM images of the individual $\text{C}_\text{ZnO}$-Dots (right). (TEM images courtesy of Dr. Mohammed J. Meziani)
in the presence of dry air. The TGA traces were shown in Figure 4.2.3 and Figure 4.2.4 according to dopants. The weight losses in the TGA traces were the major correspondents to the oxidation of carbon atoms to carbon dioxide. The remaining residues in the TGA pans after the system cooled and the pre-functionalization doped carbon nanoparticle samples (C/ZnS and C/ZnO) were identified by X-ray powder diffraction (XRD) (Figure 4.2.3 and Figure 4.2.4). These XRD results, matched with the standard XRD patterns from the JCPDS database, indicated that the existence of ZnS (Wurtzite) or ZnO (Zincite) in the pre-functionalization doped carbon nanoparticle, and the TGA residues of both C/ZnS-post and C/ZnO-post were ZnO (Zincite). These results suggest the conversion from ZnS ($M_w$ 97.5) to ZnO ($M_w$ 81.4) at high temperatures in air also contributed to the weight losses in the TGA traces of the ZnS-doped samples. Next, ZnS and ZnO contents in the doped carbon nanoparticle samples were calculated using both the weight loss within the TGA traces and the TGA residue types. These results, shown in Table 4.2.1, suggest that the ZnS or ZnO contents were little changed (within experimental error margins) between pre- and post-functionalization doped samples. The C:ZnS and C:ZnO ratios in post-functionalization samples were approximately 2:1 and 3:1, respectively, in terms of weight (corresponding to 13:1 and 19:1, respectively, in molar ratios).

The absorption and fluorescence emission (440 nm excitation) spectra of C$_{ZnS}$-Dots and C$_{ZnO}$-Dots were shown in Figure 4.2.5. The optical absorption shoulders in the blue (approximately 440 nm, Figure 4.2.5) were characteristic for both samples, where
Figure 4.2.3 TGA traces (10 °C/min in continuous air flow) for C/ZnS (A) and C/ZnS-post (B); and the X-ray diffraction pattern of C/ZnS (upper) and the TGA residue of C/ZnS-post (bottom), along with the standard ZnS (Wurtzite, ———) and ZnO (Zincite, - - -) from JCPDS database (C).
Figure 4.2.4 TGA traces (10 °C/min in continuous air flow) for C/ZnO (A) and C/ZnO-post (B); and the X-ray diffraction pattern of C/ZnO (upper) and the TGA residue of C/ZnO-post (bottom), along with the standard ZnO (Zincite) from JCPDS database (C).
Table 4.2.1 Dopant contents in the doped carbon nanoparticle samples

<table>
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<tr>
<th>Dopant</th>
<th>ZnS</th>
<th>ZnO</th>
<th>TiO$_2$</th>
<th>SiO$_2$</th>
<th>AgCl</th>
<th>Fe$_3$O$_4$</th>
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<tr>
<td>Content* (%)</td>
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<td>15%</td>
<td>10%</td>
<td>37%</td>
<td>35%</td>
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<tr>
<td>Content** (%)</td>
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<td>26%</td>
<td>16%</td>
<td>9%</td>
<td>42%</td>
<td>37%</td>
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<tr>
<td>Mole ratio (C:X)**</td>
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<td>19:1</td>
<td>35 : 1</td>
<td>51 : 1</td>
<td>26:1</td>
<td>34:1</td>
</tr>
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</table>

* in pre-functionalization samples  ** in post-functionalization samples
the excitation resulted in equally characteristic green fluorescence emissions (centered at approximately 510 nm, Figure 4.2.5) with quantum yields of around 50% and 39% (Table 4.2.2), corresponding to $\text{C}_\text{ZnS}$-Dots and $\text{C}_\text{ZnO}$-Dots, respectively.

The results of the C:ZnO ratios (Table 4.2.1), the average core diameter (Figure 4.2.2), and the UV/vis absorption and fluorescence spectra (Figure 4.2.5) of $\text{C}_\text{ZnO}$-Dots suggest there is no meaningful difference between the $\text{C}_\text{ZnO}$-Dots prepared here and previously.\(^{19}\) $\text{C}_\text{ZnS}$-Dots were synthesized through the same procedure as described previously.\(^{19}\)

As previously reported, the fluorescence from the carbon dots was associated with energy traps originating from the defect sites on the surface of carbon nanoparticles.\(^ {17}\) Though organic or polymeric molecules such as PEG\(_{1500N}\) are commonly used as passivation agents to stabilize the radiative recombination of excitons, ZnS or ZnO doping may perhaps provide more effective secondary surface passivation, resulting in brighter fluorescence emissions.\(^{19}\) With these findings, the further study was to determine if there were another dopant beyond ZnS and ZnO. Four inorganic salts, including TiO\(_2\), SiO\(_2\), AgCl, and Fe\(_3\)O\(_4\), were selected as new dopants.

The sol-gel method\(^ {23}\) was used to dope TiO\(_2\) or SiO\(_2\) on the surface of carbon nanoparticles. With the formation of pre-functionalization TiO\(_2\)-doped carbon nanoparticles (C/TiO\(_2\)) being used as an example, titanium ethoxide, ethanol, water and nitric acid were mixed, followed by refluxing at 80 °C for 1h. In this procedure, the sol-gel solution was obtained according to following reactions:
Figure 4.2.5 Absorption (ABS) and luminescence emission (FLSC, 440 nm excitation, normalized against the peak intensity) spectra of C\textsubscript{ZnS}-Dots (left) and C\textsubscript{ZnO}-Dots (right) in aqueous solutions.
Table 4.2.2 Fluorescence quantum yields of carbon dot samples in aqueous solutions at 400 nm, 440 nm and 500 nm excitation wavelengths

<table>
<thead>
<tr>
<th></th>
<th>Quantum yield (Ex 400 nm)</th>
<th>Quantum yield (Ex 440 nm)</th>
<th>Quantum yield (Ex 500 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-Dots</td>
<td>11%</td>
<td>18%</td>
<td>7%</td>
</tr>
<tr>
<td>C\textsubscript{ZnS}-Dots</td>
<td>25%</td>
<td>50%</td>
<td>12%</td>
</tr>
<tr>
<td>C\textsubscript{ZnO}-Dots</td>
<td>19%</td>
<td>39%</td>
<td>11%</td>
</tr>
<tr>
<td>C\textsubscript{TiO2}-Dots</td>
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</tr>
<tr>
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<td>2%</td>
</tr>
<tr>
<td>C\textsubscript{AgCl}-Dots</td>
<td>7%</td>
<td>12%</td>
<td>2%</td>
</tr>
<tr>
<td>C\textsubscript{Fe3O4}-Dots</td>
<td>6%</td>
<td>8%</td>
<td>1%</td>
</tr>
</tbody>
</table>
Hydrolysis: \[ \text{Ti} = \text{O} + \text{C}_2\text{H}_5 + 2\text{H}_2\text{O} \rightleftharpoons \text{Ti} + \text{OH} + 2\text{H}_2\text{O} \]
Condensation: \[ \text{Ti} + \text{OH} + \text{HO} - \text{Ti} \rightleftharpoons \text{Ti} - \text{O} - \text{Ti} + \text{H}_2\text{O} \]

When carbon nanoparticles were sonicated in the sol-gel solution, the obtained Ti condensate was adsorbed on the surface of carbon nanoparticles and then converted to a layer of TiO\(_2\) by annealing at 250 °C. The same approach and similar experimental conditions were applied to the preparation of pre-functionalization SiO\(_2\)-doped carbon nanoparticles (C/SiO\(_2\)). The functionalization of C/TiO\(_2\) or C/SiO\(_2\) with PEG\(_{1500}\) was used to derive C\(_{\text{TiO2}}\)-Dots or C\(_{\text{SiO2}}\)-Dots in an aqueous solution, which is the same procedure used to prepare the C\(_{\text{ZnO}}\)-Dots.

C\(_{\text{TiO2}}\)-Dots and C\(_{\text{SiO2}}\)-Dots were characterized by various microscopy techniques. Specifically, the TEM and AFM images of C\(_{\text{TiO2}}\)-Dots (Figure 4.2.6) and C\(_{\text{SiO2}}\)-Dots (Figure 4.2.7) suggest that the typical particle dimensions of both samples are approximately 5 nm in size. At a higher imaging resolution, the doping of carbon particles with TiO\(_2\) can be visualized. No SiO\(_2\) crystalline structure was found in the high-resolution images of C\(_{\text{SiO2}}\)-Dots. The results from energy dispersive X-ray (EDX) analyses showed the presence of C and Ti in C\(_{\text{TiO2}}\)-Dots (on a silicon grid) and Si in C\(_{\text{SiO2}}\)-Dots (on a carbon grid).

TGA and XRD were performed to measure TiO\(_2\)- and SiO\(_2\)-doped carbon nanoparticle samples. The pre-functionalization doped carbon nanoparticle samples (C/TiO\(_2\) or C/SiO\(_2\)) were dried in a vacuum oven overnight prior to measurement.
Figure 4.2.6 (A) A representative TEM (Z-contrast) image of C$_{\text{TiO}_2}$-Dots; (B) A representative AFM topography image of C$_{\text{TiO}_2}$-Dots on a mica substrate and the height profile plot along the line; (C) High-resolution TEM images of the individual C$_{\text{TiO}_2}$-Dots. (TEM images courtesy of Dr. Li Cao)
Figure 4.2.7 (A) A representative TEM (Z-contrast) image of C$_{\text{SiO}_2}$-Dots; (B) A representative AFM topography image of C$_{\text{SiO}_2}$-Dots on a mica substrate with the height profile plot along the line; (C) High-resolution TEM images of the individual C$_{\text{SiO}_2}$-Dots. (TEM images courtesy of Dr. Li Cao)
No TiO$_2$ or SiO$_2$ peaks could be identified from the XRD pattern of C/TiO$_2$ or C/SiO$_2$, respectively, which may due to the low dopant contents (~10 %) in the pre-functionalization doped carbon nanoparticle samples (Table 4.2.1). The post-functionalization doped carbon nanoparticle samples (C/TiO$_2$-post or C/SiO$_2$-post), representing the doped carbon cores in the carbon dot samples, were obtained from the aqueous solution of C$_{TiO2}$-Dots or C$_{SiO2}$-Dots by being subsequently dried by rotovap and annealed at 600 °C for 30 min to selectively remove functional groups (PEG$_{1500}$N). TGA measurements were conducted with a relatively slow heating rate of 10 °C/min to 800 °C in the presence of dry air. The TGA traces are shown in Figure 4.2.8 and Figure 4.2.9 according to dopants. The weight losses in these TGA traces were the major correspondents to the oxidation of carbon atoms to carbon dioxide, which began at approximately 400 °C for TiO$_2$-doped carbon nanoparticles but began at approximately 550 °C for SiO$_2$-doped carbon nanoparticles. The high oxidation temperature of carbon in SiO$_2$-doped carbon nanoparticles may be due to the spontaneous formation of Si-C and Si-O-C bonds in the C/SiO$_2$-interface through carbothermal reductions.$^{26}$ Subsequent to the system cooling, XRD was used to identify the remaining residues in the TGA pans (Figure 4.2.8 and Figure 4.2.9). These results, matched with the standard XRD patterns from the JCPDS database, indicated that the TGA residues for C/TiO$_2$-post and C/SiO$_2$-post were TiO$_2$ (anatase and rutile) and SiO$_2$ (no-crystalline phase), respectively. The no-crystalline phase of SiO$_2$ found in the TGA residue may perhaps be the explanation as to why no SiO$_2$ crystalline structure was observed in the high-resolution images of
Figure 4.2.8 TGA traces (10 °C/min in continuous air flow) for C/TiO$_2$ (A) and C/TiO$_2$-post (B); and the X-ray diffraction pattern of C/TiO$_2$ (upper) and the TGA residue of C/TiO$_2$-post (bottom), along with the standard TiO$_2$ (anatase, —— and rutile, - - -) from the JCPDS database (C).
Figure 4.2.9 TGA traces (10 °C/min in continuous air flow) for C/SiO$_2$ (A) and C/SiO$_2$-post (B); and the X-ray diffraction pattern of C/SiO$_2$ (upper) and the TGA residue of C/SiO$_2$-post (bottom), along with the standard SiO$_2$ (no-crystalline phase) from the JCPDS database (C).
C_{SiO2}-Dots. According to the weight losses in the TGA traces, TiO\textsubscript{2} and SiO\textsubscript{2} contents in the doped carbon nanoparticle samples were calculated as shown in Table 4.2.1. These results suggest that the TiO\textsubscript{2} or SiO\textsubscript{2} contents were little changed (within experimental error margins) between the pre- and post-functionalization doped samples. The C:TiO\textsubscript{2} and C:SiO\textsubscript{2} ratios in the post-functionalization samples were approximately 5.3:1 and 10:1 in terms of weight, respectively (corresponding to 35:1 and 51:1, respectively, in molar ratios). These values are obviously lower than the contents of dopants in ZnS- or ZnO-doped samples (Table 4.2.1), which may be due to the synthetic methods. The contents of TiO\textsubscript{2} or SiO\textsubscript{2} cannot be increased by simply increasing the usage of Ti or Si source in current sol-gel systems. In this case, dopant contents were limited by the surface adsorption ability of the carbon nanoparticles.

The absorption and fluorescence emission (440 nm excitation) spectra of C_{TiO2}-Dots and C_{SiO2}-Dots are shown in Figure 4.2.10. The absorption spectra of both samples feature a shoulder in the blue region (approximately 440 nm). Green luminescence emissions (centered at approximately 510 nm, Figure 4.2.10) were observed when C_{TiO2}-Dots or C_{SiO2}-Dots in aqueous solutions were excited at 440 nm, while C_{TiO2}-Dots exhibited a noticeably brighter fluorescence than C_{SiO2}-Dots. The fluorescence quantum yield of C_{TiO2}-Dots at 440 nm excitation was approximately 42%, a four-fold increase than that of C_{SiO2}-Dots (Table 4.2.2).

In addition to TiO\textsubscript{2} and SiO\textsubscript{2}, AgCl and Fe\textsubscript{3}O\textsubscript{4} were also selected as dopants. The pre-functionalization AgCl- and Fe\textsubscript{3}O\textsubscript{4}-doped carbon nanoparticles (C/AgCl and C/Fe\textsubscript{3}O\textsubscript{4})
Figure 4.2.10 Absorption (ABS) and luminescence emission (FLSC, 440 nm excitation, normalized against the peak intensity) spectra of C_{TiO2}-Dots (left) and C_{SiO2}-Dots (right) in aqueous solutions.
were synthesized through the same synthetic method as with C/ZnS and C/ZnO. Specifically, AgCl was formed on the surface of carbon nanoparticles through the reaction between Ag\(^+\) and Cl\(^-\) in an aqueous DMF solution, while C/Fe\(_3\)O\(_4\) was prepared by co-precipitating iron (II) sulfate and iron (III) chloride salts in an aqueous suspension of carbon nanoparticles.\(^{27}\) The identical functionalization reaction scheme and conditions were applied to the functionalization of C/AgCl or C/Fe\(_3\)O\(_4\) to yield a stable aqueous solution (C\(_{\text{AgCl}}\)-Dots or C\(_{\text{Fe3O4}}\)-Dots, respectively).

Only C\(_{\text{AgCl}}\)-Dots were characterized by using microscopy techniques. The TEM images (Figure 4.2.11) of C\(_{\text{AgCl}}\)-Dots suggest that the typical particle sizes were between 10~30 nm, with an approximate four-fold increase in size than other doped carbon dots (C\(_{\text{ZnS}}\)-Dots, C\(_{\text{ZnO}}\)-Dots, C\(_{\text{TiO2}}\)-Dots and C\(_{\text{SiO2}}\)-Dots). At a higher imaging resolution, the doping of carbon particles with AgCl are visible, with the results from energy dispersive X-ray (EDX) analyses of C\(_{\text{AgCl}}\)-Dots on a silicon grid confirming the presence of C, Ag and Cl.

TGA was performed to measure the pre-functionalization AgCl- and Fe\(_3\)O\(_4\)-doped carbon nanoparticle samples, which were dried in a vacuum oven overnight prior to measurement. The post-functionalization doped carbon nanoparticle samples (C/AgCl-post or C/Fe\(_3\)O\(_4\)-post), representing the doped carbon cores in the carbon dot samples, were obtained from the aqueous solution of C\(_{\text{AgCl}}\)-Dots or C\(_{\text{Fe3O4}}\)-Dots by being subsequently dried by rotovap and annealed at 600 °C for 30 min to selectively remove functional groups (PEG\(_{1500N}\)). TGA measurements were conducted with a relatively slow
Figure 4.2.11 TEM images of $\text{C}_\text{AgCl}$-Dots: (A) A representative S-TEM image (Z-contrast) in dark field; (B) A representative image in bright field; (C) High-resolution images of individual dots. (TEM images courtesy of Dr. Li Cao)
heating rate of 10 °C/min to 800 °C in the presence of dry air. These TGA traces, shown in Figures 4.2.12 and 4.2.13 in different dopants, exhibited weight losses that primarily correspond to the oxidation of carbon atoms to carbon dioxide. The remaining residues in TGA pans after the system cooled down were identified by XRD (Figures 4.2.12 and 4.2.13) and their XRD results were matched with the standard XRD patterns from the JCPDS database. The TGA residue for C/AgCl-post was AgCl (Chlorargyrite) mixed with the Ag metal (Silver-3C); the TGA residue for C/Fe$_3$O$_4$-post was a mixture of Fe$_2$O$_3$ and Fe$_3$O$_4$. The XRD patterns of the pre-functionalization doped samples showed there were only C and AgCl (no Ag metal) in C/AgCl, and C and Fe$_3$O$_4$ (no Fe$_2$O$_3$) in C/Fe$_3$O$_4$. We propose that AgCl decomposed at high temperature in the TGA pans to form Ag metal, while Fe$_3$O$_4$ was partially oxidized to Fe$_2$O$_3$ under the same conditions. Because of the unknown ratios between AgCl and Ag, and between Fe$_3$O$_4$ and Fe$_2$O$_3$ in the TGA residues, AgCl and Fe$_3$O$_4$ contents in the doped carbon nanoparticle samples were estimated by assuming that all of these ratios are equal to 1:1 (as shown in Table 4.2.1.) These results suggest that either the AgCl or Fe$_3$O$_4$ contents in the post-functionalization doped samples are slightly higher (within experimental error margins) than the pre-functionalization samples (Table 4.2.1). In terms of weight, the C:AgCl and C:Fe$_3$O$_4$ ratios in post-functionalization samples were approximately 1.4:1 and 1.7:1 respectively (corresponding to 26:1 and 34:1, respectively, in molar ratios).

The absorption and fluorescence emission spectra (440 nm excitation) of C$_{\text{AgCl}}$-Dots and C$_{\text{Fe3O4}}$-Dots are shown in Figure 4.2.14. The absorption spectra of both C$_{\text{AgCl}}$-
Figure 4.2.12 TGA traces (10 °C/min in continuous air flow) for C/AgCl (A) and C/AgCl-post (B); and X-ray diffraction patterns of C/AgCl (upper) and the TGA residue of C/AgCl-post (lower), along with the standard AgCl (Chlorargyrite, ——) and Ag metal (Silver-3C, - - -) from the JCPDS database (C).
Figure 4.2.13 TGA traces (10 °C/min in continuous air flow) for C/Fe₃O₄ (A) and C/Fe₃O₄-post (B); and the X-ray diffraction pattern of C/Fe₃O₄ (upper) and the TGA residue of C/Fe₃O₄-post (lower), along with the standard Fe₃O₄ (——) and Fe₂O₃ (- - -) from the JCPDS database (C).
Figure 4.2.14 Absorption (ABS) and luminescence emission (FLSC, 440 nm excitation, normalized against the peak intensity) spectra of $\text{C}_\text{AgCl}$-Dots (left) and $\text{C}_\text{Fe}_3\text{O}_4$-Dots (right) in aqueous solutions.
Dots and \( \text{C}_{\text{Fe3O4}} \)-Dots samples feature a shoulder at the same blue spectrum region, along with other doped carbon dot samples. Green luminescence emissions centered at approximately 510 nm (Figure 4.2.14) were observed when \( \text{C}_{\text{AgCl}} \)-Dots or \( \text{C}_{\text{Fe3O4}} \)-Dots in an aqueous solution were excited at 440 nm. The observed quantum yields of \( \text{C}_{\text{AgCl}} \)-Dots and \( \text{C}_{\text{Fe3O4}} \)-Dots at 440 nm excitation are approximately 7% and 6%, respectively, as shown in Table 4.2.2.

For the six types of doped carbon dots synthesized: \( \text{C}_{\text{ZnS}} \)-Dots, \( \text{C}_{\text{ZnO}} \)-Dots, \( \text{C}_{\text{TiO2}} \)-Dots, \( \text{C}_{\text{SiO2}} \)-Dots, \( \text{C}_{\text{AgCl}} \)-Dots and \( \text{C}_{\text{Fe3O4}} \)-Dots, the absorption spectra exhibited a shoulder at approximately 440 nm. The absorption spectra of these samples were normalized at 400 nm (as shown in Figure 4.2.15) along with the absorption spectrum of carbon dots (C-Dots)\(^{20}\) for comparison. The shoulder positions of carbon dot samples in their absorption spectra were slightly shifted with the application of various dopants (Figure 4.2.15). If the shoulder of C-Dots is set as a standard, \( \text{C}_{\text{ZnS}} \)-Dots, \( \text{C}_{\text{ZnO}} \)-Dots and \( \text{C}_{\text{TiO2}} \)-Dots generally exhibit larger shoulders in their absorption spectra, while \( \text{C}_{\text{SiO2}} \)-Dots, \( \text{C}_{\text{AgCl}} \)-Dots and \( \text{C}_{\text{Fe3O4}} \)-Dots show smaller shoulders. The fluorescence spectra (440 nm) of these samples were also normalized to spectral peaks as shown in Figure 4.2.16, inset for comparison. The luminescence emission spectra of both the C-Dots and doped carbon dot samples are rather similar with no significant shift or bandwidth change. However, the fluorescence brightness and corresponding quantum yields at 440 nm excitation changed dramatically when different dopants were applied (Figure 4.2.16 and Table 4.2.2). Generally, carbon dot samples with larger absorption shoulders show higher fluorescence quantum yields. Here, we name these spectroscopic changes (changes of
Figure 4.2.15 Absorption spectra (normalized at 400 nm) of C-Dots (black), C\textsubscript{ZnS}-Dots (red), C\textsubscript{ZnO}-Dots (green), C\textsubscript{TiO2}-Dots (yellow), C\textsubscript{SiO2}-Dots (blue), C\textsubscript{AgCl}-Dots (pink), and C\textsubscript{Fe3O4}-Dots (cyan) in aqueous solutions. Shown in the inset are the enlarged absorption shoulders within the blue region.
Figure 4.2.16 Fluorescence spectra (440 nm excitation) of C-Dots (black), C\textsubscript{ZnS}-Dots (red), C\textsubscript{ZnO}-Dots (green), C\textsubscript{TiO\textsubscript{2}}-Dots (yellow), C\textsubscript{SiO\textsubscript{2}}-Dots (blue), C\textsubscript{AgCl}-Dots (pink), and C\textsubscript{Fe\textsubscript{3}O\textsubscript{4}}-Dots (cyan). The emission spectral intensities are normalized to quantum yields (normalized to spectral peaks in the inset).
both absorption spectra and fluorescence brightness) in doped carbon dots as doping effects. Some of the inorganic salts (e.g. ZnS, ZnO and TiO₂) may provide positive doping effects to carbon dots, resulting in larger absorption shoulders in their absorption spectra and higher fluorescence quantum yields than that of C-Dots. Conversely, other salts such as SiO₂, AgCl and Fe₃O₄ may provide negative doping effects, resulting in small absorption shoulders and lower fluorescence quantum yields.

To further examine the doping effects for various dopants, the aqueous solutions of carbon dot samples were also excited at 400 nm. Interestingly, when the aqueous solutions of carbon dot samples were excited at 400 nm, the fluorescence emission spectra were generally broad, exhibiting two emission peaks (Figure 4.2.17). As an example, the fluorescence spectrum of C-Dots through deconvolution (resolving the curve into underlying peaks) can be represented by two overlapping Lorentzian peaks centered at approximately 465 nm and 510 nm respectively (Figure 4.2.17, inset). The ratio of area under the peak centered at approximately 510 nm to the peak centered at approximately 465 nm is \( \sim 2.7 \). The fluorescence spectra of the doped carbon dot samples were deconvoluted under the same conditions. As a result, the area ratios under the peak centered at approximately 510 nm to that centered at approximately 465 nm for C\(_{\text{ZnS}}\)-Dots, C\(_{\text{ZnO}}\)-Dots, C\(_{\text{TiO₂}}\)-Dots, C\(_{\text{SiO₂}}\)-Dots, C\(_{\text{AgCl}}\)-Dots and C\(_{\text{Fe₃O₄}}\)-Dots are equal to 155, 17, 23, 2.5, 2.5 and 1.9, respectively. Clearly, these ratios in carbon dot samples are strongly related to their fluorescence quantum yields at 400 nm excitation (Table 4.2.2), as the samples with the higher quantum yields generally show the higher ratios. The sequence
Figure 4.2.17 Fluorescence spectra (400 nm excitation) of C-Dots (black), C\textsubscript{ZnS}-Dots (red), C\textsubscript{ZnO}-Dots (green), C\textsubscript{TiO2}-Dots (yellow), C\textsubscript{SiO2}-Dots (blue), C\textsubscript{AgCl}-Dots (pink), and C\textsubscript{Fe3O4}-Dots (cyan). The emission spectral intensities are normalized to quantum yields. Shown in the inset is a deconvolution of the fluorescence spectrum of C-Dots based on two Lorentzian peaks (reproduced curve, - - -).
of quantum yields at 400 nm trends quite closely with the yields at 440 nm, as $C_{ZnS}$-Dots $> C_{TiO2}$-Dots $\approx C_{ZnO}$-Dots $> C_{AgCl}$-Dots $> C_{SiO2}$-Dots $\approx C_{Fe3O4}$-Dots.

In 2006, our group reported surface-passivated carbon dots that exhibited the featureless absorption spectra (no shoulder in the blue region) and excitation wavelength dependent emissions.\textsuperscript{17} Specifically, the excitation wavelength dependent emission spectra of carbon dot samples at 400 nm excitation exhibited one peak centered at approximately 465 nm.\textsuperscript{17,18} With slight modifications and more rigorous controls of the experimental conditions, carbon dots exhibited an absorption shoulder in the blue region.\textsuperscript{20} The excitation into the absorption shoulder (440 nm) results in a bandgap-like emission centered at approximately 510 nm. These characteristic absorption shoulder and emissions of C-Dots represent the specific electronic states/energy traps on the carbon surface known as “sweet spots”.\textsuperscript{20} Thus, in the emission spectra of the carbon dot solutions excited at 400 nm (Figure 4.2.17), we tentatively assign the emission peak centered at approximately 465 nm to the excitation wavelength dependent emission, and the peak centered at approximately 510 nm to the bandgap-like emission. It is apparent that the doping effects provided by various dopants preferentially affect the bandgap-like emissions since the samples with the higher quantum yields shows the higher ratios of area under the peak centered at approximately 510 nm to that at approximately 465 nm. For example, the bandgap-like emission of $C_{ZnS}$-Dots at 400 nm excitation almost overwhelmed the excitation wavelength dependent emission with the ratio of 155. In addition, to closely examine the doping effects to the excitation wavelength dependent
Figure 4.2.18. Fluorescence spectra (500 nm excitation) of C-Dots (black), C\(_{\text{ZnS}}\)-Dots (red), C\(_{\text{ZnO}}\)-Dots (green), C\(_{\text{TiO}2}\)-Dots (yellow), C\(_{\text{SiO}2}\)-Dots (blue), C\(_{\text{AgCl}}\)-Dots (pink), and C\(_{\text{Fe3O}4}\)-Dots (cyan). The emission spectral intensities are normalized to quantum yields (normalized to spectral peaks in the inset).
emission, various carbon dot samples were also excited at 500 nm (Figure 4.2.18), out of the blue shoulder region within the absorption spectra. At this excitation condition, the emission spectra of all samples only exhibited one peak at approximately ~535 nm, which corresponds to the excitation wavelength dependent emission (Figure 4.2.18). As shown in Table 4.2.2, fluorescence quantum yields of C-Dots and doped carbon dots at the green excitation (500 nm) retained a similar sequence as that at 400 nm or 440 nm excitation. These results suggest that the doping effects provided by inorganic salts not only affected the bandgap-like emissions, representing a larger or smaller shoulder in blue region and corresponding higher or lower emission quantum yields at 440 nm excitation according to various inorganic salts, but also affected the excitation wavelength dependent emission. These two types of emissions were either enhanced or diminished at the same time with the application of the identical dopant.

The fluorescence decays of C-Dots, C\textsubscript{ZnS}-Dots, C\textsubscript{TiO2}-Dots, C\textsubscript{SiO2}-Dots and C\textsubscript{AgCl}-Dots were measured by monitoring the emission decay using 407 nm laser pulse as the excitation source (Figure 4.2.19). While the fluorescence decays of the carbon dot samples could not be deconvoluted with a mono-exponential function,\textsuperscript{28} they could be deconvoluted with the use of a multicomponent decay function, to yield an average lifetime (τ\textsubscript{F}). For example, the fluorescence decay of C\textsubscript{ZnS}-Dots in an aqueous solution were deconvoluted well from their corresponding instrumental response functions using a triple-exponential equation to yield an average lifetime (τ\textsubscript{F}) of 5.3 ns (Figure 4.2.20 and Table 4.2.3). The average lifetimes (τ\textsubscript{F}) of C-Dots, C\textsubscript{ZnS}-Dots, C\textsubscript{TiO2}-Dots, C\textsubscript{SiO2}-Dots and C\textsubscript{AgCl}-Dots are shown in Table 4.2.3. The sequence of these lifetimes is C\textsubscript{ZnS}-Dots >
Figure 4.2.19 Luminescence decays (407 nm excitation, monitored with a 470 nm narrow bandpass filter) of C-Dots (black), C$_{ZnS}$-Dots (red), C$_{TiO2}$-Dots (yellow), C$_{SiO2}$-Dots (blue) and C$_{AgCl}$-Dots (pink). (Lifetime measurement courtesy of Dr. Li Cao)
Figure 4.2.20 Luminescence decays (407 nm excitation, monitored with 470 nm narrow bandpass filter) of C\textsubscript{ZnS}-Dots (red, \——) and the corresponding instrumental response functions (black, \——). The decay was fitted by using a triple-exponential function to yield a reproduced curve (black, \- - -). (Lifetime measurement courtesy of Dr. Li Cao)
Table 4.2.3 Fluorescence lifetimes ($\tau_F$) of carbon dot samples

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<th>Sample</th>
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<th>$\tau_{F2}$ (ns)</th>
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<td>8.3</td>
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<td>11.4</td>
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$A_1$, $A_2$ and $A_3$ are the pre-exponential factors in triple-exponential decay equation  
(Lifetime measurement courtesy of Dr. Li Cao)
C_{TiO_2}-Dots > C_{AgCl}-Dots > C-Dots > C_{SiO_2}-Dots. Generally, the fluorescence lifetimes of carbon dot samples exhibits an identical trend with that of the fluorescence quantum yields, which can be interpreted as the fluorescent species with longer lifetimes exhibit brighter emissions. The only exception was C_{AgCl}-Dots, which manifested a longer lifetime but a less emission quantum yield than that of the C-Dots. This longer or shorter lifetime of these doped carbon dots is possibly attributable to the better or worse degree of surface passivation provided by the dopants.

Mechanistically, the fluorescence in carbon dots was thought to be associated with passivated surface defects of the core carbon particles.$^{17-20}$ As such, we proposed that the additional passivation effects provided by inorganic salts may be responsible for these doping effects.$^{19}$ However, the quantum yield and lifetime results of C_{AgCl}-Dot seem to contradict our hypothesis. In comparison to C-Dots, while AgCl doping seems to provide a greater degree of surface passivation (exhibiting a longer lifetime), it also manifests a negative doping effect (a lower quantum yield). It must be emphasized that because the fluorescence species discussed here (i.e. carbon nanoparticles, inorganic salts, polymeric molecules), are very complex systems, many factors may affect their fluorescence brightness including the passivation effects. The size of the carbon nanoparticles is one of such phenomena affecting this fluorescence brightness. As previously reported, larger carbon particles with the same surface passivation were found to be much less luminescent than smaller carbon particles due to the less surface-to-volume ratios.$^{17}$ Secondly, properties of dopants may also affect the fluorescence brightness of carbon dot samples. For example, the formation of Si-C and Si-O-C bonds
in the C/SiO\textsubscript{2}-interface\textsuperscript{26} may change both the chemical structures and energy levels of the defects on the carbon surface. In addition, Fe\textsubscript{3}O\textsubscript{4} may also quench the fluorescence of carbon dots. It has been reported in literature\textsuperscript{29,30} that iron oxide nanoparticles quenched the fluorescence of semiconductor quantum dots through electronic energy transfer when packed together inside the capsules. Lastly, environmental effects cannot be ignored. For example, surfactants such as sodium dodecyl sulfate (SDS) may also play a role in these fluorescence systems. Adding a small amount of SDS (1 wt %) into an aqueous solution of C-Dots yield as much as a 20% increase in fluorescence (fluorescence quantum yields at 440 nm excitation increasing from ~17% to ~21%). The pre-functionalization doped carbon nanoparticles was dispersed in an aqueous solution of SDS and then filtrated for polymeric functionalization. The quantum yield (440 nm) of C\textsubscript{ZnS}-Dots without SDS treatment was approximately 25%, only half of the value of their counterparts that did undergo SDS treatment. Preliminary results suggest that the well dispersed nature of the particles with the aid of SDS in the aqueous solution may well be responsible for the enhanced fluorescence. With so many factors including sizes of carbon particles, properties of dopants, environmental effects and so on, the systems of doped carbon dots are too excessively complex to justify the passivation effects for each dopant according only to their fluorescence brightness (doping effects), especially for those doped carbon dot samples with poor fluorescence quantum yields (the good surface passivation is one of the requisite conditions necessary for obtaining highly fluorescent carbon dots). Nevertheless, preliminary results show that the spectroscopic performances of carbon dots (especially fluorescence quantum yields) are strongly related to the dopant type.
Even without a clear understanding of the relationship between the spectroscopic performances of carbon dots and the types of dopants, some of the doped carbon dot samples including C\textsubscript{ZnS}-Dots, C\textsubscript{ZnO}-Dots and C\textsubscript{TiO\textsubscript{2}}-Dots exhibited more than twice the fluorescence brightness than the C-Dots, suggesting that the doping by inorganic salts can be exploited as a useful contrivance for the fluorescence enhancement of carbon dots.

Since carbon dots with quantum yields nearing a range of 60% were obtained by the fractionation of as-prepared C-Dots,\textsuperscript{20} it is of considerable importance to examine how high quantum yields can be reached by fractionating these highly luminescent doped carbon dots. An aqueous gel column packed with Sephadex\textsuperscript{TM} G-100\textsuperscript{31} was used for the fractionation of C\textsubscript{ZnS}-Dots. A total of 7 fractions were collected, and the absorption and fluorescence spectra of the fractions 1, 3, 5 and 7 of C\textsubscript{ZnS}-Dots are shown in Figure 4.2.21. For all of these fractions, the absorption spectra exhibited a shoulder within the blue region that was gradually increased in later fractions. The absorption spectra of the most fluorescent fraction of C-Dot\textsuperscript{20} and the most fluorescent fraction (fraction 7) of C\textsubscript{ZnS}-Dots were subsequently compared with each other (Figure 4.2.21). The latter exhibited a larger well-defined absorption shoulder in comparison with that of the former, indicating the additional positive doping effect provided by ZnS doping. The excitation into the absorption shoulder of C\textsubscript{ZnS}-Dots in an aqueous solution results in strong luminescence in the emissions with quantum yields up to 75% which is also higher than the quantum yield record of 60% achieved from the most fluorescent fraction of C-Dots.\textsuperscript{20} Both the fluorescence quantum yields (Φ\textsubscript{F}) and lifetimes (τ\textsubscript{F}) of the varying fractions of C\textsubscript{ZnS}-Dots were plotted, the results of which are shown in Figure 4.2.22. Both quantum yields and
Figure 4.2.21 Absorption and fluorescence (440 nm excitation) spectra of the fractions 1 (A), 3 (B), 5 (C), and the most fluorescent 7 (D) of $\text{C}_{\text{ZnS}}$-Dots. Also shown in (D), represented by the dashed line, is the absorption spectrum of the most fluorescent C-Dots$^{20}$ for comparison. (Fractionation of $\text{C}_{\text{ZnS}}$-Dots courtesy of Mr. Shengtao Yang)
Figure 4.2.22 Fluorescence quantum yields (○) and lifetimes (▲) of the different fractions of C₃ZnS-Dots, and the linear relationship between the observed yields and lifetimes (inset) (Lifetime measurement courtesy of Dr. Li Cao)
lifetimes show a marked increased in later fractions. The linear relationship between the observed yields and lifetimes (Figure 4.2.22, inset) suggests a relatively uniform fluorescence radiative process throughout the fractions (namely, that the observed fluorescence quantum yield variations were due to a predominant change in the competing nonradiative processes from fractions to fractions). The fluorescence radiative rate constants \( k_F = \Phi_F / \tau_F \) of C\(_{\text{ZnS}}\)-Dots fractions exhibited an average \( \sim 1 \times 10^8 \text{s}^{-1} \), indicating very strong electronic transitions and large molar absorptivities of these samples at the blue absorption band. These results from the fractionation of C\(_{\text{ZnS}}\)-Dots (including both the linear relationship between observed quantum yields and lifetimes, and the fluorescence radiative rate constants) are similar to those results obtained previously from the fractionation of C-Dots.

The most fluorescent fraction of C\(_{\text{ZnS}}\)-dots was characterized by using microscopy techniques. As shown in Figure 4.2.23, the most fluorescent fraction represented well-dispersed carbon dots with an average size of 5.1 nm which exhibits no major difference with the pre-fractionation samples, except those fractions containing, on average, slightly smaller particles, and a narrower distribution according to statistical analyses. At a higher imaging resolution, the doping of a carbon particle with ZnS can be seen. (Figure 4.2.23)

The identical fractionation strategy was applied to the fractionation of the C\(_{\text{TiO}_2}\)-Dots composed of 7 sample fractions. The absorption and fluorescence spectra of the fractions 1, 3, 5 and 7 of C\(_{\text{TiO}_2}\)-Dots are shown in Figure 4.2.24. The subsequent fractions exhibited larger absorption shoulders and higher luminescence quantum yields, which are similar to the fractionation results of C\(_{\text{ZnS}}\)-Dots. The quantum yields of the most
Figure 4.2.23 A representative TEM image of the most fluorescent C$_{ZnS}$-Dots (upper) and high-resolution images of two individual dots (lower), with the corresponding statistical size analysis results based on multiple images. (TEM images courtesy of Dr. Li Cao)
Figure 4.2.24 Absorption and fluorescence (440 nm excitation) spectra of fractions 1 (A), 3 (B), 5 (C), and the most fluorescent 7 (D) of $C_{TiO_2}$-Dots. (Fractionation of $C_{TiO_2}$-Dots courtesy of Mr. Shengtao Yang)
Figure 4.2.25 Fluorescence quantum yields (○) and lifetimes (▲) of the different fractions of C_{TiO2}-Dots, and the linear relationship between the observed yields and lifetimes (inset). (Lifetime measurement courtesy of Dr. Li Cao)
fluorescent fraction of $C_{TiO2}$-Dots were able to reach 69%. Fluorescence quantum yields ($\Phi_F$) and lifetimes ($\tau_F$) of the varying fractions of $C_{TiO2}$-Dots are plotted in Figure 4.2.25. Generally, the quantum yields and lifetimes in varying fractions have retained their linear relationship (similar to, but not as perfect as indicated by the results from the fractionation of $C_{ZnS}$-Dots). The fluorescence radiative rate constants ($k_F={\Phi_F}/\tau_F$) of $C_{TiO2}$-Dots fractions, as well as the values of C-Dots$^{20}$ and $C_{ZnS}$-Dots fractions, were on average $\sim 1 \times 10^8 \text{ s}^{-1}$.

The mechanism of fluorescence enhancement of C-Dots$^{20}$ or doped carbon dots by the fractionation is not yet fully understood. Later fractions with higher fluorescence quantum yields and longer fluorescence lifetimes, however, may be elucidated by the greater degree of surface passivation provided by the surface functional group (PEG$_{1500N}$) together with the influence from the smaller particle size.$^{20}$ Beyond this hypothesis, it can be concluded that both the doping and the gel-column fractionation may enhance the fluorescence brightness of carbon dots. While ZnS currently provides the best doping effects, the fractionation by gel column can be used to obtain the most-fluorescent PEG$_{1500N}$ passivited carbon dots. Hence a combination of both of these effects can result in a quantum yield of near 75% for the most fluorescent fraction of $C_{ZnS}$-Dots under study. It is also important to realize that neither the surface doping nor procedures of the gel-column fractionation were optimized to the best conditions. Thus far, the potential spectroscopic performances of carbon dots may be perhaps close to or even within reach of quantitative fluorescence emissions (Quantum yield = 1).
In summary, inorganic salts can provide additional either positive or negative doping effects to carbon dots. Experiment results showed that the fluorescence brightness of these carbon dots was significantly enhanced by ZnS, ZnO or TiO$_2$ doping. In combination with the gel-column fractionation, the most fluorescent fractions of doped carbon dots can reach quantum yields of 75%. Further studies should focus upon optimizing the conditions of the surface doping and the gel-column fractionation, along with experiments toward understanding of these highly fluorescent carbon dot samples.
References


CHAPTER FIVE

FLUORESCENCE DECORATION OF DEFECTS IN CARBON NANOTUBES

5.1 Introduction

Carbon nanotubes exhibit many interesting and/or unique properties, including those that may be exploited for optical applications.\textsuperscript{1-7} For example, the optical absorption of semiconducting single-walled carbon nanotubes (SWNTs) is characterized by transitions associated with the van Hove singularities in the electronic density of states.\textsuperscript{6,8} The corresponding bandgap fluorescence in the near-IR has been studied extensively for various purposes from fluorescence bioimaging to optically “sorting” SWNTs according to their diameters.\textsuperscript{9-11}

The well-dispersed and functionalized carbon nanotubes, either single- (SWNTs) or multiple-walled (MWNTs), were also found to be fluorescent in the visible spectral region, extending into the near-IR.\textsuperscript{5,12} The emissions are thought to be derived from nanotube surface defects, for which the functionalization likely provides the necessary passivation effects.\textsuperscript{12} Interestingly, the results from fluorescence polarization spectroscopy suggested that the absorption and fluorescence emission transitions are parallel,\textsuperscript{13} obviously along the nanotube axis according to a separate fluorescence excitation study of the functionalized nanotubes in an anisotropic polymer host (mechanically stretched polymer film).\textsuperscript{14} Similar to the bandgap fluorescence, the surface defect-derived emissions are also subject to significant inter-tube quenching effects in nanotube bundles.\textsuperscript{12} Thus, defect-derived fluorescence has been used as an indicator for
the effectiveness of nanotube dispersion and functionalization,\textsuperscript{15,16} such as in the study of carbon nanotubes as fillers in polymeric nanocomposite materials.\textsuperscript{16}

Surface defects in carbon nanotubes have been considered in a number of studies for their potentially major effects on some of the important nanotube properties.\textsuperscript{1,17-19} Because they are defect-derived, visible fluorescence emissions in functionalized carbon nanotubes may be used for the probing and understanding of the defects. Moreover, the defect sites in carbon nanotubes may be exploited to derive other interesting properties. Here we report that the surface defects in carbon nanotubes can be decorated by an inorganic salt, followed by organic functionalization (Scheme 5.1), to result in significantly enhanced visible fluorescence emissions. The surface decoration and the associated strong fluorescence may potentially serve as markers for the structural defects in carbon nanotubes.

5.2 Experimental Section

5.2.1 Materials

SWNTs (carbonaceous purity of the sample 40-60\%) were purchased from Carbon Solutions, Inc., and MWNTs (nanotube purity 95\%) from Nanostructured & Amorphous Materials, Inc. The samples were purified according to procedures already reported in the literature.\textsuperscript{20} Zn(CH\textsubscript{3}COO)\textsubscript{2}·2H\textsubscript{2}O (>98\%) and Na\textsubscript{2}S·9H\textsubscript{2}O (>98\%) were obtained from Alfa, and \textit{O,\textit{O}}'-bis(3-aminopropyl) polyethylene glycol (MW ~ 1,500, PEG\textsubscript{1500N}) from Fluka. \textit{N,N}-dimethylformamide (DMF, 99\%) and sodium dodecyl sulfate
Scheme 5.1 Representation of PEG$_{1500}$N-functionalized ZnS-coated carbon nanotube
(SDS, 99%) were supplied by Acros and VWR, respectively. Millipore Durapore membrane filters (pore size 0.22 µm) were acquired from Fisher, dialysis membrane tubing from Spectrum Laboratories, and holey carbon-coated copper grids from Electron Microscopy Sciences. Water was deionized and purified by being passed through a Labconco WaterPros water purification system.

5.2.2 Measurements

Centrifuge (Baxter megafuge model 2630, up to 6,000g) and bath sonicator (VWR model 250D) were used. Thermogravimetric analysis (TGA) was performed on a TA Instruments Q500 TGA analyzer. Transmission electron microscopy (TEM) images were obtained on Hitachi 9500 TEM and Hitachi HD-2000 S-TEM systems, with the latter also being used for the energy dispersive X-ray spectroscopy (EDX) analysis. Atomic force microscopy (AFM) imaging was carried out in the acoustic AC mode on a Molecular Imaging PicoPlus system equipped with a multipurpose scanner and a NanoWorld Pointprobe NCH sensor.

UV/vis absorption spectra were recorded on a Shimadzu UV2101-PC spectrophotometer, and fluorescence spectra on a Spex Fluorolog-2 emission spectrometer equipped with a 450 W xenon source and a detector consisting of a Hamamatsu R928P photomultiplier tube operated at 950 V. Fluorescence quantum yields were determined by the relative method, with quinine sulfate as a fluorescence standard. Fluorescence imaging of surface-dispersed samples was performed on a Leica laser scanning confocal fluorescence microscope (DMIRE2, with Leica TCS SP2 SE scanning
system) equipped with an argon ion laser (JDS Uniphase) and a femtosecond pulsed
(~100 fs at 80 MHz) Ti:Sapphire laser (Spectra-Physics Tsunami with a 5 W Millennia
pump). An oil immersion objective lens (Leica X63/1.40) was used in both one- and two-
photon imaging experiments, while an external non-descanned detector (NDD) was used
for the two-photon fluorescence measurement. Raman spectra were obtained on a
Renishaw Raman spectrometer equipped with a 50 mW diode laser source at 785 nm
excitation.

5.2.3 ZnS-Coated Nanotubes and Functionalization

The purified SWNTs (600 mg) were dispersed into DMF (300 mL) via sonication
(720 W, 50 kHz) for 30 min. Zn(CH$_3$COO)$_2$·2H$_2$O (680 mg, 3.1 mmol) was added to the
suspension under vigorously stirring, followed by slow dropwise addition of aqueous
Na$_2$S (0.62 M, 5 mL). The precipitate containing ZnS-coated SWNTs (SWNT/ZnS) was
washed repeatedly with water and then dried in a vacuum oven at 60 °C. The same
procedure was applied to the preparation of the sample containing ZnS-coated MWNTs
(MWNT/ZnS).

A portion of the sample containing SWNT/ZnS (150 mg) was dispersed in an
aqueous SDS solution (1 wt%, 120 mL) via sonication for 30 min. Upon filtration, the
filter cake was washed repeatedly with water, dried, and then mixed thoroughly with
PEG$_{1500N}$ (1.5 g). The mixture was heated to 110 °C and stirred for 72 h under nitrogen
protection. It was then cooled to room temperature and dispersed in water, followed by
centrifuging at 3,500 g to retain the supernatant as a solution of PEG$_{1500N}$-funcitonalized
SWNT/ZnS (PEG-SWNT/ZnS). The same functionalization reaction scheme and conditions were applied to the preparation of PEG-MWNT/ZnS.

For the functionalization of the carbon nanotubes without ZnS coating (for various comparisons), purified SWNTs (200 mg) were mixed with PEG_{1500N} (2 g) at 110 °C, and the mixture was stirred at that temperature for 72 h under nitrogen protection. It was then cooled to room temperature and dispersed in water, followed by centrifuging at 3,500g to retain the supernatant as a solution of PEG_{1500N}-functionalized SWNTs (PEG-SWNT). Similarly, the PEG_{1500N}-functionalized MWNTs (PEG-MWNT) were prepared.

5.3 Results and Discussion

The coating of carbon nanotubes with ZnS was achieved in the formation reaction for the insoluble zinc salt, where the suspended nanotubes served as nucleation centers. TGA analyses of the coated nanotube samples SWNT/ZnS and MWNT/ZnS were performed with a relatively slow heating rate (10 °C/min) to 800 °C in air to determine the ZnS contents in the samples. Under the TGA conditions, the nanotube carbons were oxidized into carbon dioxide (thus purged out of the system), while the ZnS converted into ZnO as residue. The ZnS-to-C mole ratios thus determined were around 1-to-16 in both SWNT/ZnS and MWNT/ZnS samples.

The ZnS-coated nanotube samples were functionalized in the classical amidation reaction with the amino-PEG (PEG_{1500N}) to yield aqueous soluble PEG-SWNT/ZnS and PEG-MWNT/ ZnS. Similar TGA analyses of the functionalized samples suggested that
the ZnS-to-C (nanotube carbons only) ratios were little changed (within experimental error margins) from those of the pre-functionalization samples.

Like the PEG\textsubscript{1500N}-functionalized nanotubes without ZnS coating (denoted as PEG-SWNT and PEG-MWNT), the PEG-SWNT/ZnS and PEG-MWNT/ZnS samples were readily soluble in water and resulted in colored aqueous solutions (stable, free from any precipitation). The solutions were diluted for the dispersion of each sample onto a piece of cover-glass. The resulting specimens of PEG-SWNT/ZnS and PEG-MWNT/ZnS on the cover-glass substrate were examined using a confocal fluorescence microscope. Interestingly, fluorescence images for both specimens exhibited predominantly elongated species with bright spots separated by less emissive segments (Figure 5.1). For comparison, the PEG-functionalized SWNTs and MWNTs without the ZnS coating (PEG-SWNT and PEG-MWNT, respectively) were imaged under the same conditions. In those images similarly elongated yet only weakly emissive species could be identified; however, no bright spots were observed (Figure 5.1).

The obviously much brighter fluorescence emissions in the samples with the ZnS coating were confirmed in solution-phase measurements. As compared in Figure 5.2, the observed fluorescence intensities of the ZnS-coated samples in solution are much higher than those without the coating (quantum yields around 16-19% for the former vs 2-3% for the latter, in reference to quinine sulfate as fluorescence standard\textsuperscript{22}), though the spectral features are similar. Figure 5.2 also demonstrates that the fluorescence spectra of the coated samples measured on the imaging platform under the fluorescence microscope are in general agreement with those obtained in solution, confirming the correspondence
Figure 5.1 Fluorescence images (458 nm excitation at about 100 mW) of the specimens on cover glass, left: PEG-SWNT/ZnS (upper), PEG-MWNT/ZnS (lower), and insets for corresponding selected species at a higher resolution; right: PEG-SWNT (upper) and PEG-MWNT (lower). The measurement conditions were about 7 µs integration time per pixel and 2 s scanning time per image.
Figure 5.2 Absorption (ABS) and fluorescence (440 nm excitation) spectra, upper: PEG-SWNT/ZnS (——) and PEG-SWNT (- - - -); lower: PEG-MWNT/ZnS (——) and PEG-MWNT (- - - -). Shown in insets are corresponding comparisons between fluorescence spectra of the specimens (──o──) and those in solution (——).
between the bright fluorescence images for the coated nanotubes on the cover glass substrate (Figure 5.1) and their intense solution-phase fluorescence emission spectra. Based on these results, the ZnS coating must be responsible for the observed significant enhancement in fluorescence properties of the coated samples. Also like in functionalized carbon nanotubes without the ZnS coating, the observed fluorescence emissions were highly photostable, exhibiting no significant decreases in intensities over repeated excitations.

Mechanistically, fluorescence emissions in functionalized carbon nanotubes have been attributed to the presence and passivation (as a result of the functionalization) of nanotube surface defects, which act as emissive energy trapping sites upon photoexcitation.\textsuperscript{5,12} In recent studies of fluorescence from functionalized small carbon nanoparticles, a similar surface passivation mechanism was proposed for the observed fluorescence emissions.\textsuperscript{23} Interestingly, it was also found that the coating of the small carbon nanoparticles with an inorganic salt like ZnS or ZnO before organic surface functionalization could result in much brighter fluorescence.\textsuperscript{24} The enhancement there was attributed to ZnS or ZnO coating the particle surface sites and augmenting the passivation effectiveness of the organic functional molecules. Here in the case of carbon nanotubes, the ZnS might be coating the nanotube surface defects (including the broken nanotube ends) and similarly contributing and augmenting the passivation of the defect sites by the PEG molecules (Scheme 5.1). Therefore, the role of ZnS coating in the functionalized carbon nanotubes is essentially fluorescence decoration, in which the
surface defects on nanotubes are decorated for much brighter fluorescence emissions (Figure 5.1).

It might be tempting to assign the elongated species in the confocal images (Figure 5.1) to individual nanotubes and the bright spots to defect sites on the nanotubes. However, these species in the images were probably too large in size to be associated with only individual SWNTs or MWNTs. More likely, these were nanotubes in small bundles coated by ZnS or/and small bundles of ZnS-coated nanotubes, all functionalized by PEG molecules. The kind of bundling in the latter should not be negative to fluorescence emissions (hardly favorable to intertube quenching). Even in the former the attachment between two nanotubes in a bundle might be associated with only a small tube segment in each nanotube, thus consistent with the overall “tree branch-like” configurations observed as dominant features in fluorescence images (Figure 5.1). In fact, the brighter spots in the “tree branch-like” images (Figure 5.1) were likely due to the PEG-functionalized nanotube surface defects that were also heavily coated by ZnS (Scheme 5.1). It should be pointed out that the ZnS nanoparticles themselves had no absorption at the excitation wavelength (458 nm) used for the fluorescence imaging,25 and thus they could not be responsible for the observed fluorescence emissions. The bright fluorescence images must be due to the functionalized carbon nanotubes (with ZnS coating).

For higher spatial resolution, the same specimen was characterized by AFM in such a way that the area on the specimen selected for imaging at a higher resolution by AFM would approximately match that used in the fluorescence imaging. As shown in
Figure 5.3, the matching of the imaging area between the two techniques was successful, which allowed the AFM imaging results to provide more nanoscale structural information on the species responsible for the fluorescence images. In the AFM phase image (Figure 5.3), there are obvious contrasts between the hard (nanotubes and ZnS) and soft (PEG molecules) materials, due to their different interactions with the AFM tip. It might be argued that the unevenness in the AFM profiles for the hard materials was consistent with the ZnS coating of the nanotubes. The hard materials in the AFM image, especially those that appeared uneven, generally matched well with the bright fluorescent species in the confocal image (Figure 5.3). Again, the fluorescence images must be associated with the carbon nanotubes, with the brighter spots likely corresponding to the areas or segments with significant ZnS coating.

The coating of carbon nanotubes by ZnS nanoparticles was visualized in electron microscopy imaging. The specimens of PEG-SWNT/ZnS and PEG-MWNT/ZnS for the imaging were prepared by depositing a dilute solution of each sample onto a holey carbon-coated copper grid, followed by evaporation and drying. As shown in Figure 5.4, the ZnS nanoparticles were not evenly distributed along the nanotube, but instead aggregated around some sections of the nanotube (more clearly illustrated in the case of PEG-MWNT/ZnS as the larger size of MWNT made the TEM imaging easier). The presence of ZnS in those selected sections was confirmed by results from the energy dispersive X-ray (EDX) analysis on the same imaging platform. It might be argued that these sections “attracting” ZnS nanoparticles were probably more defective, though such an argument could not be verified unambiguously in terms of the electron microscopy
Figure 5.3 The PEG-MWNT/ZnS specimen on cover glass: the AFM phase (upper left, with the highlighted regions enlarged in middle left), height (lower left) images, fluorescence image at 458 nm excitation (upper right, enlarged from the highlight region in the inset), and the overlapping between the AFM phase and fluorescence images (lower right).
imaging alone. Nevertheless, at a higher imaging resolution, the ZnS nanoparticles were found to be on the nanotube surface, and both the nanoparticles and carbon nanotubes were covered by soft materials (Figure 5.4), consistent with the functionalization by the amino-PEG molecules. The results from the special AFM-confocal matching experiments and the electron microscopy characterization were all consistent with the structure illustrated in Scheme 5.1 for the elongated fluorescent species with periodic brighter spots found in the confocal images (Figure 5.1). Apparently for the visible fluorescence derived from passivated surface defects in functionalized carbon nanotubes, the coating of the defects with ZnS could result in significant enhancements in the fluorescence properties. This is phenomenologically and probably also mechanically similar to what has been observed and reported for fluorescent carbon nanoparticles,\textsuperscript{23,24} namely that the effect of ZnS coating in both functionalized carbon nanotubes and nanoparticles might be attributed to the contribution of the ZnS to defect or surface passivation, in addition to what is provided by the attached PEG molecules. The enhanced passivation effect by the ZnS coating for brighter fluorescence emissions is relatively easier to probe in carbon nanotubes than in carbon nanoparticles since the one-dimensional structure of the former provides a better imaging platform with fluorescence confocal microscopy.

Mechanistic details on the fluorescence enhancement by ZnS coating of the nanotube defects remain to be understood. However, a clear distinction should be made between the defects-derived fluorescence emissions discussed here and those associated with bandgap transitions.\textsuperscript{9-11} In fact, the bandgap fluorescence in semiconducting SWNTs is quenched or even diminished by the presence of defects and their exaggeration (such as
Figure 5.4 TEM images of the specimens on holey carbon-coated copper grids, upper: PEG-SWNT/ZnS (left: S-TEM Z-contrast dark field; right: TEM bright field; and insets: corresponding high-resolution images); lower: PEG-MWNT/ZnS (left and right: S-TEM Z-contrast dark field; and inset: TEM bright field at high resolution).
chemical functionalization at the defect sites). For the effect of ZnS coating, a reviewer proposed an interesting possibility that the coating might have increased the population of defect sites rather than enhanced the defect passivation. However, according to previous studies the fluorescence emissions were generally associated with passivated structural defects, not noncovalent modification of nanotube walls. Raman results of ZnS-coated carbon nanotubes obtained from thermal defunctionalization (heated to 400 °C and kept for 30 min in inert atmosphere) of PEG-SWNT/ZnS and PEG-MWNT/ZnS are shown in Figure 5.5. The larger D bands (1336 cm⁻¹ for SWNT/ZnS and 1311 cm⁻¹ for MWNT/ZnS) than G bands (1563 cm⁻¹ for SWNT/ZnS and 1594 cm⁻¹ for MWNT/ZnS) suggested a high population of defects in the functionalized samples. However, there were reports in the literatures showing no meaningful changes in resonance Raman results between carbon nanotubes without and with ZnS coating, implying no significant increases in the defect population post ZnS coating. Thus, the preferential solubilization of carbon nanotubes containing more defect sites in the functionalization reactions may be accounted for the high population of defects.

The bright visible fluorescence emissions in PEG-SWNT/ZnS and PEG-MWNT/ZnS samples could also be observed with two-photon excitation in the near-IR. In the measurements, the same specimens on the cover glass substrate used in the confocal imaging were excited at 800 nm with a femtosecond pulsed laser. The fluorescence images monitored by an external nondescanned detector on the confocal
Figure 5.5 Raman spectra (785 nm excitation) of SWNT/ZnS (-----) and MWNT/ZnS (· · ·) obtained from thermal defunctionalization (heated to 400 °C and kept for 30 min in inert atmosphere) of PEG-SWNT/ZnS and PEG-MWNT/ZnS, respectively. (Raman spectra courtesy of Mr. Sushant P. Sahu)
microscope exhibited similarly tree branch-like features with some brighter spots (Figure 5.6), in general agreement with those found in the confocal imaging (Figure 5.1). The two-photon (800 nm excitation) fluorescence spectrum for the specimen on the microscope also matched well with the solution-phase spectrum measured in a fluorescence spectrometer (400 nm excitation, Figure 5.6), suggesting that the same visible fluorescence emissions could be obtained by either one- or two-photon excitation. In summary, the surface defects in carbon nanotubes upon functionalization exhibit visible fluorescence emissions. The effect of functionalization may be augmented by coating the defects with an inorganic salt such as ZnS to result in much enhanced fluorescence intensities, which may prove valuable to applications that rely on the optical properties of carbon nanotubes. The fluorescence decoration with the coating may also serve as a tool in the study of surface defects in carbon nanotubes. The observation of similarly enhanced two-photon fluorescence emissions in the coated carbon nanotubes may also prove significant, as in general brightly two-photon fluorescent dyes are scarce. Further structural and mechanistic investigations on these nanomaterials are needed and planned.

(Chapter 5 has been published in the literature as reference 30.)
Figure 5.6 Two-photon (800 nm femtosecond laser excitation) fluorescence image of PEG-MWNT/ ZnS (left) and a comparison of the corresponding fluorescence spectrum (─o─) with the one-photon spectrum in solution (400 nm excitation, ———).
References


APPENDICES

Coauthored Publications during My Graduate Study


vivo.” *Small* **2008**, *4*, 940.


Effective Purification of Single-Walled Carbon Nanotubes with Reversible Noncovalent Functionalization

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An effective purification method for single-walled carbon nanotubes (SWNTs) based on a combination of oxidative and reversible noncovalent functionalization with 1-pyrene carboxylic acid is reported. The functionalization was selective toward the nanotubes, allowing a nearly complete removal of residual metal catalysts and carbonaceous impurities. The resulting highly pure SWNTs retained solvent-dispersible, a valuable feature to potential applications that require solvent-based processing. The functionalization agent could be recovered quantitatively and reused. Effects of the purification process on the composition and properties of the nanotube sample were evaluated.

Introduction

Single-walled carbon nanotubes (SWNTs) have been investigated extensively for their unique properties and many potential applications. For SWNTs from all three principal production techniques (arc discharge, laser ablation, and chemical vapor deposition), the as-produced samples contain a significant amount of metal catalysts (nickel—yttrium for those from the arc discharge production) and various carbonaceous impurities (amorphous carbon, nanocrystalline graphite, etc.). Therefore, effective purification methods are required for the removal of these impurities. Among the various purification methods available in the literature, a relatively simple and popular procedure is based on the treatment with oxidative acid, typically aqueous nitric acid under refluxing condition, though the purity of the resulting sample is still limited.9–12 For SWNTs of higher purity, a combination with other treatments has found some success.13–18

For example, Haddon and co-workers demonstrated recently that the acid-treated sample could be further purified by centrifugation of aqueous dispersions, either high-speed directly or low-speed at an acidic pH.13,15 In an alternative approach, Georgakilas et al. exploited the selectivity of the 1,3-dipolar cycloaddition reaction to SWNTs for the purpose of purification.14 The nanotubes were functionalized with azomethine ylides in DMF for their solubilization, thus being separated from impurities in the sample, followed by thermal annealing to remove the functional groups for the recovery of purified SWNTs. These authors reported that the sample from the functionalization and recovery scheme was mostly free of metal particles, but still with significant carbonaceous impurities.18

In this work, we combined the oxidative acid treatment with reversible noncovalent functionalization of arc discharge-produced SWNTs for their aqueous solubilization, which allowed the effective removal of residual metal catalysts and carbonaceous impurities in centrifugation. The resulting highly pure SWNTs remained solvent-dispersible, similar to those from only the oxidative acid treatment, thus requiring no changes to the experimental protocols already available in the literature for the use of the nanotubes in devices and various other applications.

Experimental Section

Materials. 1-Pyrene carboxylic acid (97%) was obtained from Aldrich and used as received. Concentrated hydrochloric acid (HCl, 37%) was purchased from Acros, sodium hydroxide (NaOH, 99%) from Sigma, N,N-dimethylformamide (DMF) and ethyl acetate from Mallinckrodt Baker, and PVDF membrane filters (0.22 μm pore size) from Fisher Scientific. Water was deionized and purified by being passed through a Labconco WaterPro water purification system.

The SWNT samples from the arc discharge method were supplied by Carbon Solutions, Inc., and were also produced in-house on a classical arc discharge setup. The samples from the different sources were generally quite similar, with carbon contents up to 60%.

**Measurements.** Fisher Scientific centrifuge (model 128) and homogenizer (PowerGen 125) and VWR bath sonicator (model 220D) were used in the purification experiments. Thermogravimetric analysis (TGA) was performed on a TA Instruments Q500 thermogravimetric analyzer. Optical absorption spectra were recorded on a Shimadzu UV-2600 UV/visible-IR spectrophotometer. Raman spectra were obtained on a Jobin Yvon T6400 Raman spectrometer equipped with a Motan-Cortix-120 laser (35 mW) source for 632.8 nm excitation, a triple monochromator, an Olympus BX-41 microscope, and a liquid-nitrogen-cooled symphony detector. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images and energy-dispersive X-ray (EDX) results were acquired on a Hitachi HD-2000 scanning-TEM system.

**Purification.** A SWNT sample (1 g) was first heated in an oven at 300 °C in the presence of air for 30 min, followed by refluxing in a concentrated nitric acid (2.5 M, 500 mL) for 24 h. After being cooled to room temperature, the mixture was filtered, washed with deionized water repeatedly until neutral pH, and then dried in a vacuum oven to yield the purified sample (0.6 g).

A portion of the above-prepared SWNT sample (150 mg) and 1-pyreneacetic acid (200 mg) were added to an aqueous NaOH solution (0.1 M, 100 mL), and the mixture was homogenized for 30 min and then sonicated for 12 h. The resulting suspension was centrifuged at 1300 g for 30 min to retain the dark-colored supernatant. The supernatant was acidified to pH ~3 with an aqueous HCl solution for precipitation of the nanotubes. Upon filtration with PVDF membrane filter (0.22 µm pore size), the collected SWNT sample was carefully washed with deionized water and then ethyl acetate in repeated dispersion–centrifugation cycles, followed by refluxing in ethyl acetate for 12 h. The purified SWNTs (105 mg) were recovered from the precipitate and 1-pyreneacetic acid from the solution nearly quantitatively.

**Results and Discussion**

The TGA trace for an as-supplied or as-produced SWNT sample in the presence of air (continuous air flow) showed residual weight close to 40% (Figure 1) due primarily to metal catalysts used in the arc discharge production and their corresponding oxides. The gradual weight loss in approximately the 600–750 °C temperature range of the same TGA (Figure 1) was for the oxidation of the residual graphite (the carbon precursor in the arc discharge production) into carbon dioxide. The aqueous nitric acid treatment removed a substantial amount of metal catalysts, reducing the weight of final residue (Figure 1). However, contents of both residual metal catalysts and graphite were still significant in the nitric acid-treated sample, as suggested by TGA (Figure 1) and electron microscopy results (Figures 2 and 3).

In the nitric acid-purified sample, the SWNTs could be functionalized and solubilized by 1-pyreneacetic acid in a basic aqueous solution. The solubilization was apparently discriminative against carbonaceous impurities and also those nanotubes that are encapsulated or associated with metal catalysts, resulting in their poor dispersion and thus precipitation in the

![Figure 1. TGA traces (10 °C/min in continuous air flow) for the as-produced (dotted line), nitric acid-treated (dashed line), and further 1-pyreneacetic acid purified (solid line) samples of SWNTs.](image1)

![Figure 2. Representative SEM images for the as-produced (top), nitric acid-treated (middle), and further 1-pyreneacetic acid purified (bottom) samples of SWNTs.](image2)
centrifugation (1300 g). On the other hand, the dark-colored supernatant of the functionalized SWNTs in basic aqueous solution remained stable for as long as the observation period (at least 3 months). The mode of functionalization was likely associated with π-π interactions of the pyrene and the SWNT surface, and the aqueous solubility of the functionalized SWNTs was made possible by the conversion of pyreneacetic acid into pyreneacate under the basic condition. During the course of this work, Ajayan and co-workers reported the use of similar pyrene derivatives for the noncovalent functionalization of SWNTs.26

The same functionalization chemistry was apparently less effective toward the impurities, which were removed as precipitates. The selectivity in terms of the functionalization effectiveness between the nanotubes and impurities served as the basis for the purification approach.

The functionalized SWNTs were soluble only in basic aqueous solution due to the pyreneacate form discussed above, but precipitated quantitatively upon acidification of the solution. The removal of 1-pyreneacetic acid from the nanotube surface and other salts from the sample was accomplished via repeated washing with water and ethyl acetate, including refluxing in ethyl acetate. According to UV/vis absorption results, the functionalization agent 1-pyreneacetic acid was recovered nearly quantitatively (more than 98%) in the washing process and could be reused. The SWNTs free from 1-pyreneacetic acid were characterized by using several established techniques.

At the bulk sample level, the TGA trace clearly suggested that the SWNTs were very pure, without any significant weight loss due to residual graphite precursor (Figure 1). The final residue, due predominately to residual metal catalysts and their oxides, was much less than that in the nitric acid-treated sample (prepurification with 1-pyreneacetic acid). Under the assumption that the metal catalysts were all converted to oxides at high temperatures during the TGA scan, for which the oxygen content of the pyrolysate products must be subtracted from the final residue,12,20 the purified sample of SWNTs should contain no more than 3% of residual catalysts by weight according to the TGA result, obviously much less by volume because of the much higher density of the metals than that of carbon.

The electron microscopy imaging results allowed a more direct examination of the purified SWNTs (Figures 2 and 3). The specimens for SEM and TEM were prepared by first dispersing the purified sample in DMF and then depositing a few drops of the dispersion onto a carbon-coated copper grid, followed by the evaporation of the solvent. The SEM image for the purified sample showed SWNTs in bundles of 5–20 nm in diameter and up to several micrometers in length, largely free from the impurities (Figure 2), in contrast to those in SEM images for the residual sample removed in the purification with 1-pyreneacetic acid and also the sample treated with nitric acid only (Figure 3).

The metal particles as residual catalysts are much higher in terms of electron density than carbon in the nanotubes, so their presence in the specimen could be detected sensitively by TEM imaging in the Z-contrast mode. The absence of such particles in Z-contrast images (Figure 3) must be due to their low population in the sample, consistent with the effectiveness of the purification method. The EDX analysis detected nickel and yttrium only in the specimens from the residual sample removed in the purification with 1-pyreneacetic acid, as well as the sample treated with nitric acid only (Figure 3).

The intrinsic optical and electronic properties of SWNTs were preserved in the purification via reversible noncovalent functionalization with 1-pyreneacetic acid. Thin films of the purified sample were prepared for characterization by optical absorption and resonance Raman spectroscopy. As shown in Figure 4, the

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The absorption spectra of SWNTs before (upper) and after (lower) purification with reversible noncovalent functionalization with 1-pyrenecarboxylic acid are shown in Figure 4. These spectra feature characteristic absorption bands of SWNTs corresponding to the first and second pairs of van Hove singularities in the electronic density of states for semiconducting SWNTs, with prominent bands at 1530 and 2000 cm\(^{-1}\), respectively. The absorption of metallic SWNTs at 725 nm could also be observed (Figure 4). These absorption features are characteristic of SWNTs from the arc discharge production, but little change from those found in the simply nitric acid-treated sample (Figure 4).

There were no significant changes in the resonance Raman spectra of SWNTs from the as-supplied or as-produced sample to the very pure sample, still featuring the radial breathing mode at 1450 cm\(^{-1}\), a weak D-band at 1355 cm\(^{-1}\), a G-band at 1500 cm\(^{-1}\), and a D\(^\prime\)-band at 2626 cm\(^{-1}\). The as-supplied or as-produced sample of SWNTs is a mixture of metallic and semiconducting nanotubes, and the resonance Raman spectra of metallic and semiconducting SWNTs are different. Therefore, the observed Raman spectra are sensitive to the composition of metallic and semiconducting nanotubes in the sample. Since a different hydrophobic pyrene derivative was used previously for the separation of metallic and semiconducting SWNTs, an interesting issue in this work was whether the 1-pyrenecarboxylic acid purification would alter the relative populations of metallic and semiconducting SWNTs. According to results from repeated careful Raman measurements, the spectra of the as-supplied or as-produced sample and the very pure sample from the 1-pyrenecarboxylic acid purification were consistently the same (Figure 5), suggesting that 1-pyrenecarboxylic acid under the experimental conditions for purification would not affect the original composition of metallic and semiconducting SWNTs. The further purification of the nitric acid-treated sample with reversible noncovalent functionalization by 1-pyrenecarboxylic acid did not change in any significant way the dispersibility of the nanotubes in water or polar organic solvents (DMF, for example). The preservation of dispersibility was important to the characterization of the purified SWNTs, such as the fabrication of thin films for optical absorption measurements (Figure 4), and will likely be highly valuable to those applications that require solvent-based processing.

The results from this work suggested that the widely used and relatively simple nitric acid treatments could remove most of the metal catalysts from the arc discharge production, but less so with respect to graphitic impurities. The purification with 1-pyrenecarboxylic acid improved substantially in both. More quantitatively, estimated yields of the purification processes, the nitric acid treatment left behind close to 60% of the starting as-supplied sample, from which the 1-pyrenecarboxylic acid purification yielded about 70%. Therefore, the combined purification captured most of the SWNTs in the as-supplied sample.

Mechanistically, the noncovalent functionalization with 1-pyrenecarboxylic acid must be favorable to the well-structured SWNTs, discriminative against carbonaceous impurities such as graphitic particles and broken or highly defective nanotubes. For those nanotubes encapsulated with residual metal catalysts, they may either structurally too defective as a result or too heavy with the encapsulated metals and oxides, thus unfavorable to the noncovalent functionalization or solubilization, respectively. All of these served as the basis for the observed effective purification.

In conclusion, the use of 1-pyrenecarboxylic acid in basic aqueous solution is an effective purification method for simple nitric acid-treated sample to yield highly pure yet still solvent-dispersible SWNTs. The quantitative recovery and ease of the purification against 1-pyrenecarboxylic acid represent an important feature valuable to larger-scale applications of the purification method.

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Carbon Dots as Nontoxic and High-Performance Fluorescence Imaging Agents

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Fluorescent carbon dots (small carbon nanoparticles with the surface passivated by oligomeric PEG molecules) were evaluated for their cytotoxicity and in vivo toxicity and also for their optical imaging performance in reference to that of the commercially supplied CdSe/ZnS quantum dots. The results suggested that the carbon dots were biocompatible, and their performance as fluorescence imaging agents was competitive. The implication to the use of carbon dots for in vitro and in vivo applications is discussed.

Introduction

Fluorescent nanoparticles, primarily semiconductor quantum dots (QDs), have attracted much recent attention for a variety of purposes and applications, especially for their potential use as optical imaging agents.1-9 Among extensively investigated and currently well-established QDs are those based on cadmium selenide (CdSe) in various particle sizes, and their core–shell configurations (CdSe/ZnS in particular) for improved performance. These QDs are widely considered as being more advantageous over conventional organic dyes as well as genetically engineered fluorescent proteins in terms of the optical brightness and, thus, imaging sensitivity, photostability, resistance to metabolic degradation, etc.4 For example, CdSe/ZnS QDs have been demonstrated for superior performance in a number of in vitro and in vivo optical imaging experiments.10-17 As widely acknowledged, however, a major issue for the QDs containing cadmium or other heavy metals is toxicity. There are existing data suggesting that these QDs are toxic to vertebrate systems at relatively low concentrations,18-21 with risks for their accumulation in organs and tissues.22-24 It was also reported recently that the QDs could induce acute toxicity and proembolic effect in mice.25,26

The search for benign (nontoxic) alternative QD-like fluorescent nanomaterials has continued. Of particular interest and significance was the recent finding that small carbon nanoparticles could be surface-passivated by organic or inorganic compounds to become strongly fluorescent in the visible and near-infrared spectral regions.26 These surface-functionalized carbon nanoparticles ( dubbed “carbon dots”, Figure 1 ) were found to be physisorptionally and photochemically stable and nonleaching, thus different from the semiconductor QDs.26-31 Carbon dots (C-Dots) also exhibited very high two-photon absorption cross sections, enabling fluorescence imaging with both one- and two-photon excitations on the same platform.32 There is growing evidence suggesting that C-Dots represent an emerging new class of QD-like fluorescent nanomaterials for applications in optical bioimaging33 and beyond.

Carbon has generally not been considered as a toxic element, hardly in the same category as cadmium and other heavy metals discussed above. However, for the specific material configurations and structures found in C-Dots, there are legitimate concerns on their biocompatibility in vitro and in vivo, especially in light of the recent results and controversies on the toxicity issues of fullerenes and carbon nanotubes.34,35

Figure 1. Top: A cartoon illustration of C-Dots. Bottom: A representative AFM topography image of C-Dots on mica (with a height profile plot along the line).
Here we report results from in vitro and in vivo evaluations confirming that C-Dots are, indeed, nontoxic and also results from optical imaging in terms of fluorescence microscopy suggesting that C-Dots are competitive in performance to the well-developed (commercially available) CdSe/ZnS QDs.

Results and Discussion

The C-Dots (small carbon nanoparticles surface-passivated by PEG1000) Figure 1 were prepared in slightly modified procedures reported previously. For the same C-Dots with the carbon core significantly enriched with 13C (denoted as 13C-Dots), a carbon target from a mixture of 12C powder and graphite cement was laser-ablated to yield the 13C-enriched carbon nanoparticles, for which the same surface passivation chemistry with PEG1000 was used to produce the 13C-Dots. TEM and AFM (Figure 1) results suggested that the C-Dots and 13C-Dots were around 4–5 nm in average diameters, similar to those reported previously.

These PEGylated (with PEG1000) C-Dots were found to be strongly fluorescent both in aqueous solution (quantum yield about 20% at 440 nm excitation) and on a substrate under single dot-like conditions (via near-infrared laser excitation). In contrast, C-Dots were known to be nonfluorescent (9). As shown in Figure 2, the green fluorescence images of individual C-Dots were generally bright, comparable to those of the commercialized CdSe/ZnS QDs (aqueous compatible Qdot 525 ITK amino (PEG) QDs from Invitrogen) under the same excitation, detection, and other instrumental conditions. In a more statistically meaningful calibration for the fluorescence brightness of C-Dots against that of the CdSe/ZnS QDs, all dots (fluorescent spots) in multiple images of each sample were sorted in terms of their relative intensities, which amounted to about 1,200 dots for each sample (Figure 2). The brightness distributions of the two samples thus obtained, while not different in any substantial fashion, suggested that the C-Dots sample was less homogeneous in terms of fluorescence brightness at the individual dot level. However, it should be cautioned that the observed brightness variation might also be subject to effects from some measurement issues, such as some dots being slightly out of the focal plane in a specific imaging experiment on a specimen containing many dots.

The C-Dots were similarly fluorescent with two-photon excitation (femtosecond pulsed laser excitation at 800–880 nm). Thus, two-photon fluorescence images of the same specimen used in the confocal imaging above were collected (Figure 2). The one- and two-photon fluorescence imaging results of C-Dots were generally comparable, as reported previously. For the commercially supplied CdSe/ZnS QDs (aqueous compatible Qdot 525 ITK amino (PEG) QDs from Invitrogen), however, the same two-photon excitation conditions and also some variations around those conditions failed to yield similarly bright fluorescence images. Since it was reported that CdSe/ZnS QDs could be strongly two-photon fluorescent, a simple explanation might be that these commercially supplied QDs were not optimized for such a purpose.

The toxicity evaluations of C-Dots in vitro were based on their effects on the proliferation, mortality, and viability of human breast cancer MCF-7 and human colorectal adenocarcinoma HT-29 cells. For both cell lines, all these parameters were little affected by C-Dots, no more than those by the surface passivation agent PEG1000 (Figure 3). The carbon core in C-Dots is similar to free carbon nanoparticles, which in various nanoscale configurations have not been found to pose any significant toxic effects. PEG1000 at a high concentration could be toxic to cells, since PEG molecules have been used as
fisogen in cell membrane diffusion. Nevertheless, the concentrations of C-Dots used in the in vitro evaluations were significantly higher than those required for potential applications such as optical imaging of living cells, so were the much longer exposure times. Thus, C-Dots should be considered as nontoxic for typical cellular experiments, especially in comparison with PEGylated CdSe/ZnS or CdSe/CdS QDs, for which cytotoxicity has been cited as a significant concern in the literature. For example, a cell viability loss of more than 25% was observed for human epidermal keratinocytes after exposure to the PEGylated CdSe/ZnS QDs at 10 μM for 24 h, or a 50% loss for prostate renal proximal cells after similar exposure to PEGylated CdSe/CdS QDs.\textsuperscript{29}

CD-1 mice were used for the toxicity evaluation of C-Dots in vivo. The mice in two groups were exposed to two different dosages of C-Dots, 8 and 40 mg carbon core-equivalent/kg body weight, and the third group exposed to 0.9% NaCl aqueous solution was taken as the nontoxic control. All of the mice exposed to C-Dots or NaCl solution survived during the 4 weeks of the experiment. The food intake was regular, and no mice exhibited any symptom of anorexia, or other clinical symptoms such as hair loss, scab, vomiting, or diarrhea. The activities of the exposed mice were normal, without any violent or lethargic behavior. The body weight increase was a general indicator were similar among the three groups.

Established serum biochemistry assays were used to evaluate more quantitatively the influence of C-Dots on the exposed mice, including especially those for potential hepatic injury and kidney functions. As shown in Figure 4, the two important hepatic indicators, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were at similar levels for the mice exposed to different dosages of C-Dots and the control. The three indicators for kidney functions, uric acid (UA), blood urea nitrogen (BUN), and creatinine (Cr), were also unchanged among the three groups of mice (Figure 4). These results suggest no toxicity of C-Dots in mice at exposure levels beyond those commonly used in optical imaging in vivo and for relatively long exposure times (up to 28 days). In any case, the toxicity of C-Dots, if any, is considerably lower than that of CdSe-based QDs, for which detectable toxic effects were reported to be at exposure down to the picomole level.\textsuperscript{10} The C-Dots are particularly amenable to in vivo uses because PEG molecules (such as PEG\textsubscript{12000} used in the surface passivation) are biocompatible and nontoxic in vivo, in contrast to their cytotoxicity at high concentrations.

The organs of the mice postexposure to C-Dots at the higher dosage (40 mg carbon core-equivalent/kg body weight) were harvested for histopathological analyses. As shown in Figure 5, the structures of organs from the exposed mice were normal, hardly different from those of the control group. There was no steatosis, necrosis, or hydropic degeneration in the exposed hepatic sections. Typical splenic exud and lymphocytic were observed clearly in the spleen sections (Figure 5). Similarly, in the sections of kidneys, the glomerulus structure could be distinguished easily. No protein liquid or necrosis was found in all groups. As found for other carbon nanomaterials, the amounts of C-Dots in liver and spleen were relatively much higher than those in other organs, but still low in absolute population. For
example, in specimens of dissected liver and spleen harvested 6 h postexposure of C-Dots (about 20 mg carbon core-equivalent/kg body weight injected), a few scattered bright fluorescence spots were detected (Figure 5), attributable to trapped C-Dots. Separately according to isotope-ratio mass spectroscopy (isotope-MS) analyses$^{36,37}$ of the liver and spleen samples from mice exposed to $^{13}C$-Dots, the carbon core-equivalent contents were on the order of 20 mg and 2 mg in liver and spleen, respectively. Both the fluorescence imaging and isotope-MS results suggest relatively minor accumulations of C-Dots in vivo, consistent with the absence of any meaningful damage to the organs, though more systematic investigations on in vivo biodistributions of various carbon dots are still needed and being pursued. Nevertheless, the imaging results here also serve to demonstrate the kind of sensitivity that might be expected from C-Dots in potential fluorescence bioimaging in vivo.

It seems evident that in terms of biocompatibility, the advantage of C-Dots over the CdSe-based QDs is overwhelming. In addition, the biocompatibility of C-Dots is also competitive with some currently FDA-approved dyes as optical imaging agents. For the fluorescent dye indocyanine green, as an example, the LD$_50$ (median lethal dose) to intravenously exposed mice is 60 mg/kg body weight.$^{35}$ Although a rigorous determination of the LD$_50$ for C-Dots has yet to be accomplished, similar performance at least in the same order of magnitude may be expected.

The optical performance of C-Dots is generally compatible to that of the aqueous compatible CdSe/ZnS QDs, where the higher absorptivity of C-Dots compensates for the lower average fluorescence quantum yield. However, the C-Dots sample is less homogeneous in terms of performance by individual dots, presenting opportunities to potentially isolate the brighter dots for much improved optical properties. Another significant advantage of C-Dots over the QDs is the smaller size, less than 10 nm vs more than 20 nm in diameter, respectively, making C-Dots more suitable for tracing small proteins or probing fine biological structures.$^{38,39}$ The smaller size also allows minimal injection volume in potential in vivo applications.

In summary, the oligomeric PEG-functionalized carbon dots were evaluated in vitro and in vivo, from which the results suggested that these fluorescent dots are nontoxic to the selected cell lines (no more than that of the oligomeric PEG molecules); neither do they impose any significant toxic effects on the mice at dosages beyond those commonly used for in vivo optical imaging. In addition to their apparent biocompatibility, the carbon dots exhibited competitive (on the order of magnitude, at least) fluorescence imaging performance to that of the commercially supplied CdSe/ZnS QDs, demonstrating their potentials for both in vitro and in vivo applications.

Experimental Section

C-Dots and $^{13}C$-Dots. The preparation of precursor carbon nanoparticles and the synthesis of carbon dots were based on the previously reported procedures,$^{10}$ with slight modifications and more rigorous controls of the experimental conditions for improved fluorescence properties. Briefly, the carbon soot was refluxed in aqueous microparticle solution (2.6 M) for 12 h, dialyzed against fresh water, and then centrifuged at 10000g to retain the supernatant. The recovered sample was refluxed in neat SOCl$_2$ for 1 h. Upon the removal of excess SOCl$_2$, the sample (100 mg) was mixed well with carefully dried PEG$_{8000}$ (1 g) in a flask, heated to 110 °C, and vigorously stirred under nitrogen protection for 3 days. The reaction mixture at room temperature was dispersed in water, and centrifuged at 2500g to retain the supernatant, followed by filtration through a Sephadex G-100 (GE Healthcare) column (4.8 cm x 15 cm) to keep the colored fraction. The $^{13}C$-Dots were similarly prepared by starting with the $^{13}C$-enriched carbon soot (from laser ablation of a highly $^{13}C$-enriched graphite target$^{3,4,34}$).

Fluorescence Imaging. A Leica laser scanning confocal fluorescence microscope (DM IRE2, with Leica TCS SP2 SE scanning system) equipped with an argon ion laser (JDS Uniphase) and a frequency-doubled pulsed Ti:sapphire laser (Spectra-Physics Tsunami) with a 5 W Millennia pump) was used for all measurements. Both the C-Dots and commercially supplied CdSe/ZnS QDs (Invitrogen Qdot 525 FITC amino (PEG) QDs) were diluted to 5 m M in deionized water. Each 5 µL aliquot was dropped onto a glass slide, followed by drying in air. The specimens (dots dispersed on slides) were imaged under the same instrumental conditions (one-photon, 450 nm excitation and 470–820 nm emission collection; two-photon, 800–880 nm excitation and 455–750 nm emission collection). The images were processed and analyzed with NIH Image software.

For the imaging of dissected liver and spleen, the harvested organs were fixed in 4% formaldehyde solution, embedded in paraffin, and then thin-sectioned. The sections were mounted on glass microscope slides by using the standard histopathological techniques without staining.

Assays in vitro. MCF-7 and HT-29 cells were cultured using established procedures. In the 96-well plates, MCF-7 cells were plated at 2 × 10$^4$ cells per well, and HT-29 cells at 1 × 10$^5$ cells per well. After incubation for 24 h, the C-Dots with a carbon core-equivalent concentration of 1, 5, 10, 20, 50, 100, or 200 µg/ml (diluted to the final exposure concentrations with the culture medium just prior to the cell exposure) and PEG$_{8000}$ were introduced to cells. Cells cultured in the free medium were taken as the control. Another 24 h later, the cytotoxicity was examined according to well-established protocols. The cell mortality (positive cell number/total cell number in percentage) was evaluated by counting the trypan blue positive cells, as was the cell proliferation (total cell number of the exposed group/total cell number of the control group in percentage). The cell viability was evaluated by the MTT assay (measuring the ability of mitochondrial reduction of the tetrazolium salt MTT to formazan by succinic dehydrogenase).

Evaluations in vivo. Male CD-1 mice (~25 g) were acquired and used at Beijing University in compliance with the institutional Animal Care and Use Program Guidelines. The mice were cared for by following established protocols. After acclimation, the mice were randomly divided into groups of five each for the in vivo toxicity evaluations. Each mouse was exposed intravenously to C-Dots in a single injection of 200 µg carbon core-equivalent in 200 µl, or three injections (with 4 h intervals between injections) of 333 µg in 333 µl each. Mice exposed to 6.9% NaCl aqueous solution were taken as the control group.

At 1, 7, and 28 days postexposure, mice were sacrificed, and blood and organ samples were collected for toxicological assays. Serum samples were obtained from blood by centrifugation (3000 rpm x 10 min). Organ samples were cut off and fixed in 4% formaldehyde solution. All biochemical assays were performed on a Hitachi 7170A clinical autoanalyzer. ALT (U/L), AST (U/L), UA (mmol/L), BUN (mmol/L), and Cr (µmol/L) were measured by using commercial kits (Bühlmann Laboratories, Switzerland). For histopathological observation, the thin-sectioned tissue specimens were stained with hematoxylin and eosin (H&E) and examined under light microscopy.
Organs harvested from the mice exposed to $^{13}$C-Doses were
homogenized and lyophilized for quantification in terms of the
$^{13}$C isotopic abundance by using isotope-ratio mass spectrometry
(Thermo Finnigan DELTA$^{13}$ XP), for which detailed protocols for
measurements and data processing are already available in the
literature.30-31

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Advances in Bioapplications of Carbon Nanotubes

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This progress report provides an overview on recent advances in bioapplications of carbon nanotubes including the chemical modification of carbon nanotubes, targeting specifically their covalent and noncovalent conjugations with a variety of biological and bioactive species (proteins and peptides, DNAs/RNAs, and carbohydrates). Furthermore, the significant recent development and progress in the use of carbon nanotubes for biosensors, drug and other delivery systems, bioimaging, etc. and in the understanding of in vivo biodistribution and toxicity of carbon nanotubes are reported.

1. Introduction

Carbon nanotubes are pseudo-one-dimensional carbon allotropes of high aspect ratio, high surface area, and excellent material properties such as ultimate electrical and thermal conductivities and mechanical strength. While these all-carbon hollow graphite-like nanomaterials may be conceptually be viewed as “rolled up” structures of one or more layers of graphene sheets for single-walled (SWNT) or multi-walled (MNT) carbon nanotubes, respectively, their formation is generally spontaneous in various production methods. There have been major advances in the availability of carbon nanotubes, both in quality and quantity, which has in turn stimulated the worldwide pursuit of carbon nanotubes for technological applications. Nevertheless, carbon nanotubes (especially SWNTs) are still relatively expensive, so that their use in higher-value but lower-quantity technologies are probably better justified in the near term. As one of such technological areas of enormous potential, bioapplications of carbon nanotubes have attracted much attention, with significant recent progress generating much excitement in the research community.

The unique properties of carbon nanotubes offer a wide range of opportunities and application potential in biology and medicine. For example, the rich electronic properties of carbon nanotubes have been explored for the development of highly sensitive and specific nanoscale biosensors. Promising results have been produced on the use of carbon nanotubes in various electroanalytical nanotube devices and as electromechanical actuators for artificial muscles. The optical absorption of carbon nanotubes in the near-infrared has been used for the laser heating cancer therapy. In recent years, the rapid development toward maturation of methodologies for the chemical modification and functionalization of carbon nanotubes has helped to address many bio-compatibility-related issues, opening up an even wider range of bioproduction opportunities in areas such as drug delivery, biocojugation and specific recognition, including interestingly the use of sugar-functionalized carbon nanotubes to bind and aggregate anthrax spores. The biological fate and consequences of carbon nanotubes in vitro and in vivo have also been investigated for an understanding of the important issues on environmental impact and toxicity.

In an earlier report, we highlighted some of the available methodologies for the aqueous dispersion and solubilization of carbon nanotubes and discussed some representative results on modifications of carbon nanotubes with various biological and bioactive species. Along the same line, here we report the recent advances in the chemical modification of carbon nanotubes targeting specifically their bioapplications, review various approaches and developments toward the use of carbon nanotubes in biology and medicine, and provide some perspectives on future investigations and emerging opportunities.

2. Functionalization of Carbon Nanotubes

There has been significant recent progress in the development and implementation of various covalent and noncovalent functionalization methods for chemical modification and solubilization of carbon nanotubes. The functionalization that has been achieved in the functionalization and especially the aqueous solubilization of carbon nanotubes is particularly beneficial to the biocompatibility of the nanotubes toward bioprocesses. Among now widely used covalent functionalization approaches are addition reactions derived from those traditionally for graphite surfaces or established for fullerences (Scheme 1A), and coupling reactions targeting oxidized defect sites on the carbon nanotube surface, commonly the surface defect-derived carboxylic acid moieties to react with amines and alcohols for amide and ester linkages, respectively (Scheme 1B).
For some functional groups such as amines, the covalent addition functionalization is likely accompanied by significant noncovalent interactions or the adsorption of the functional groups on the nanotube surface, thus substantially improving some properties of the functionalized nanotube samples such as excellent aqueous solubility for a hydrophilic functionalization agent\(^{[24]}\). Noncovalent functionalization approaches are equally important to the biocompatibility and bioapplications of carbon nanotubes.\(^{[12]}\) Among commonly used schemes are hydrophobic or π–π interactions. Because it is noncovalent, the functionalization does not disrupt the conjugated electronic structure in nanotubes, though the strong interactions may still significantly affect the electronic transitions in the nanotubes (often considered as surface doping effects).\(^{[13]}\) The functionalization procedures are generally straightforward, and a variety of functionalization agents including surfactants, polymers, polymeric aromatic compounds, and biomolecules have been used for the noncovalent dispersion of carbon nanotubes. For example, dispersions of mostly individual SWNTs have been obtained by using sodium dodecyl sulphate (SDS) under ultrasonication-ultracentrifugation conditions\(^{[10]}\) for nearly complete optical assignments of SWNT\(^{[10]}\) and for further chemical manipulation of individual nanotubes.\(^{[26]}\) Like SDS, many other anionic, cationic, and non-ionic surfactants are also capable of dispersing individual SWNTs. However, the amount of nanotubes dispersed by SDS or other surfactants is generally small (on the order of 5% or less)\(^{[21]}\). The use of polymers instead of surfactants has not improved the dispersion efficiency in any substantial fashion.\(^{[21]}\)

Chemical modification and functionalization of carbon nanotubes for their dispersion and solubilization have evolved...
into a rather broad research field. Highlighted below are more specifically some recent advances in the modification and functionalization of carbon nanotubes with biological or biocompatible molecules or systems.

2.1. Proteins and Peptides

Proteins could be conjugated with carbon nanotubes via either spontaneous adsorption onto the nanotube surface or the immobilization more controllably in functionalization reactions. For example, Nepal and Gerleker demonstrated that common proteins such as lysozyme, histone, hemoglobin, myoglobin, ovalbumin, bovine serum albumin, trypsin, and glucose oxidase were good dispensing agents for SWNTs and that the ability to disperse these proteins depended on various factors including the primary structure and pH.[29] Proteins with more basic residues (histone and lysozyme) were more effective in the dispersion of SWNTs. For pH effect in the functionalization of SWNTs with hemoglobin, either a low (acidic) or high (basic) pH was required for enhanced solubilization.[30]

In a study by Pooritzsch et al., the dispersion of SWNTs with amphiphilic helical peptides,[31] the effectiveness of the peptides was found to be dependent on the electron density of the aromatic residue on the hydrophobic face. There has also been evidence on the selectivity of proteins towards metallic SWNTs, which was proposed to be potentially useful in the separation of as-produced nanotube samples (previously mixtures of metallic and semi-conducting SWNTs).[32,33]

Sun and coworkers functionalized SWNTs covalently with bovine serum albumin (BSA) or horse spleen ferritin in classical carbodiimide-activated amidation reactions.[34,35] The resulting BSA-functionalized SWNTs were coated with goat anti-Escherichia coli O157 to form immuno-SWNTs under physiologically compatible conditions.[36] These immuno-SWNTs were able to recognize pathogenic E. coli O157:H7 cells through specific antibody-antigen interactions (conjugate without the antibody as a negative control), as confirmed by imaging results from scanning electron microscopy (SEM) (Fig. 1).[37] The bacterial recovery determined by colony enumeration was up to 80-90% when a relatively larger amount of immune-SWNT and/or a relatively higher initial bacterial count were used.[37]

Similarly, Kase and coworkers functionalized MWNNTs covalently through an oligomeric ethylene glycol linker with a specific peptide sequence which binds to the heparinase receptor-binding subunit of anthrax toxin.[38] The nanotube-peptide conjugate selectively destroyed the anthrax toxin due to the nanotube-mediated generation of reactive oxygen species following near-IR irradiation.[39]

The immuno-nanotube approach was expanded with the introduction of magnetic components, namely the preparation of immunomagnetic carbon nanotubes. Sun and coworkers isolated BSA-functionalized magnetic MWNNTs (those encapsulated with iron and/or iron oxides) via magnetic separation, and then coated them with anti-E. coli O157. The resulting immunomagnetic MWNNTs were used for the immunomagnetic separation of pathogenic E. coli O157:H7 in pure and mixed (with Salmonella typhimurium) cultures.[40] The immunomagnetic separation was quite efficient, with the bacteria in aqueous buffer being captured by the immunomagnetic MWNNTs and precipitated in a commercial magnetic separator within 5 min. The colony enumeration revealed that the sensitivity for such capture of bacteria was approximately 40 bacterial counts per 0.1 mL aqueous buffer. The capture was also highly selective, with no bacterial background bacteria even at a relatively high interference concentration (selective toward E. coli O157:H7 at 800 colony forming units (CFU)/0.1 mL with background flora of S. typhimurium DT104 cells at ~3 1000 CFU/0.1 mL).[41]

2.2. DNAs/RNAs

Similar to proteins, DNAs and RNAs could be attached to carbon nanotubes noncovalently by adsorption[42-45] and covalently in carbodiimide-activated coupling reactions either directly[P45,46] or through a bifunctional linker.[47] For example, Hamers and coworkers used the bifunctional linker approach to functionalize sidewalls of carbon nanotubes in several steps, starting by the reaction of a 4-nitrobenzenesulfonyl azide, then the electrochemical reduction of NO₂ to NH₂ for reaction with a heterobifunctional cross-linker to introduce a maleimide group, followed by coupling with 5'-thiol-modified single strand DNA (ssDNA).[48] The nanotube-bound ssDNAs were able to recognize their appropriate complementary sequence with a high degree of selectivity.

The specific interaction between DNA sequences grafted on the carbon nanotube surface was used in the assembling of nanotubes into architectures necessary for electrical circuits and molecular sensing applications. In an exploration on DNA-directed multicomponent self-assembly of carbon nanotubes and gold nanoparticles,51-53 ssDNA was first grafted onto the nanotube surface via direct wrapping or carbodiimide-activated coupling to form ssDNA-SWNT conjugates and then hybridized
with complimentary DNA sequences grafted (via the specific thiol-gold interaction) on gold nanoparticles. The DNA-directed assembly was found to be reversible: formation at low temperature and dissociation at high temperature.[33] The DNA-SWNT approach has also been used in probing the surface defect sites of carbon nanotubes. In a recent study,[34] amine-terminated ssDNA sequences were covalently attached to the broken tips and sidewall defects of SWNTs (via the amination of defect-derived carboxylic acids), followed by their binding with gold nanoparticles that were modified with complimentary DNA sequences, where the gold nanoparticles served as readily detectable probes.

The functionalization (or wrapping) of carbon nanotubes with DNA not only imparts aqueous solubility but also allows a more precise control of the interface properties. For example, Dai and coworkers adsorbed poly-T DNA noncovalently (via π-stacking of the base-pairs) onto SWNT sidewalls to enable ultrathin dielectrics for nanotube electronics.[35] As reported, the DNA functionalization made it possible to fabricate SWNT field effect transistors (FETs) free of gate-leakage for potentially ultimate performance. The same group also grafted short interfering RNA (siRNA) onto the carbon nanotube surface, where the thiol-modified siRNA cargo molecules were linked to aqueous solubilized SWNTs (functionalized with an amine-terminated surfactant) through cleavable disulfide bonds.[36] According to results from in vitro delivery experiments, these siRNA-SWNT conjugates could effectively silence (50-60% knockdown) both CD4 receptor and CXCR4 co-receptor which are required for HIV to enter into human T-cells and infection.

The nanotube-bound DNAs have been shown to largely retain their biological activities. Luciferase DNA containing T7 promoter sequence, were adsorbed onto SWNT `back-yard', and ~30% of the bound DNA remained functional as compared to the native (solution-based) DNA in RNA polymerase-catalyzed transcription/translation reaction.[37] The interaction between DNA and carbon nanotubes is also generally stable. In fact, the stability has recently allowed the separation of the dispersed nanotubes into well-defined subpopulations.[38,39] The ssDNA-wrapped SWNTs were separated into multiple fractions, each with a different length distribution, and the fractions were examined for their cellular toxicity with different cell lines.[40] According to the competitive cell uptake assays, SWNTs above a length threshold (~200 nm) were excluded from the cells, whereas those below the threshold were able to access the cell interior, significantly decreasing the metabolic activity, namely that shorter SWNTs could be more toxic to cells than longer ones.[41]

The mechanism on the DNA wrapping of carbon nanotubes is not fully understood, though a number of driving forces have been discussed,[42,43] such as van der Waals and hydrophobic (π–π stacking) interactions, the entropy loss due to the confinement of the DNA backbone, and electronic interactions between DNA and carbon nanotubes. These have also been studies of DNA-nanotube conjugates and the associated conformational change of DNA on the nanotube surface by a combination of molecular dynamic simulation, circular dichroism, and optical spectroscopy.[44–46] It was found that the double-stranded DNA noncovalently wrapping SWNTs underwent conformational transition from right-handed B form to the left-handed Z form upon the addition of divalent cations such as Hg(II), corresponding to a detectable red shift in the band-gap emission of SWNTs (Fig. 2). Thus, these DNA-wrapped nanotubes were used to detect ions in whole blood, tissue, and within living mammalian cells.[46]

![Figure 2](image_url)  
**Figure 2.** A) Concentration-dependent fluorescence response of the DNA-encapsulated (6,5) nanotube to divalent cation concentrations. The inset shows the (6,5) fluorescence band at starting (blue) and final (pink) concentrations of Hg(II). B) Fluorescence energy of DNA-SWNTs inside a dialysis membrane upon removal of Hg(II) during a period of 7 h by dialysis. (C) Circular dichroism spectra of unbound (--) and DNA at various concentrations: of Hg(II). D) DNA-SWNT emission energy plotted versus Hg(II) concentration (red curve) and the ellipticity of the 285-nm peak obtained via circular dichroism measurements upon addition of mercuric chloride to the same oligonucleotide (black curve). Arrows point to the axis used for the corresponding curve. (E) Illustration of DNA undergoing a conformational transition from the B form (top) to the Z form (bottom) on a carbon nanotube. Reproduced with permission from [45]. Copyright 2006 American Association for the Advancement of Science.
2.3. Carbohydrates

Another advantageous use of the unique pseudo-one-dimensional structure of carbon nanotubes has been in the multivalent display of carbohydrate ligands for various biological functions and interactions. Carbohydrates are highly hydrophilic biomolecules and used extensively in the functionalization of carbon nanotubes through either covalent bonding or noncovalent wrapping. The nanotube-displayed carbohydrates not only impart significant aqueous solubility and biocompatibility, but also offer the kind of bioactivities that are apparently not available with other displaying platforms such as polymeric nanoparticles. For example, Sun and coworkers recently demonstrated that the mannosaccharide (galactose or mannose) functionalized SWNTs could effectively bind and aggregate anthrax (Bacillus anthracis) spores in the presence of a divalent cation like Ca\(^{2+}\), with the reduction in CFU reaching 97.7% as a result of the aggregation. Interestingly, however, the polymeric nanoparticles (polystyrene beads or diameter) functionalized with the same sugars exhibited no similar binding and aggregation of the spores under the same experimental conditions, suggesting the uniqueness of SWNT as a linear and semi-flexible scaffold for multivalent displaying of the monosaccharides. The Ca\(^{2+}\)-mediated binding and aggregation of B. anthracis spores were found to be reversible, a complete de-aggregation of the spores when free Ca\(^{2+}\) was removed by adding the chelating agent ethylene diamine tetraacetic acid (EDTA). Therefore, mechanistically the binding was probably a result of divalent cation-mediated carbohydrate-carbohydrate interactions between the SWNT-displayed multivalent monosaccharides and the sugar moieties on the spore surface.

The binding of galactose-functionalized SWNTs (Gal-SWNT) with pathogenic E. coli O157:H7 cells required no divalent cation mediation, resulting in significant cell agglutination. The same behavior was not found with mannose- or BSA-functionalized SWNTs, namely that the binding and agglutination were specific to the nanotube-displayed galactose ligands. This could be due to the presence of galactose-binding proteins on the E. coli cell surface. It is well-established in the literature that the multivalency is required for any effective carbohydrate-carbohydrate or carbohydrate-protein interactions. Interestingly, however, when galactose molecules were displayed in pairs in the sugar dendron-functionalized carbon nanotubes (thus, in principle, enhanced carbohydrate multivalency), their binding with the pathogenic E. coli for cell agglutination was improved even marginally.

The sugar functionalization and solubilization of carbon nanotubes could also be achieved via noncovalent wrapping with polysaccharides, for example, Liu et al. used alginate acid (AA), a natural polysaccharide constituted with repeated β-1,4-N-linked acid (M) and α-1,4-galacturonic acid (G) segments, to stabilize MWNTs by sonication. Results from transmission electron microscopy (TEM) and NMR analyses of the AA-MWNT complex suggested that the nanotubes were partially wrapped by AA in a configuration possibly with the M segments lying down and the G segments standing up on the nanotube surface. The addition of divalent or trivalent metal cations, such as Zn\(^{2+}\), Ni\(^{2+}\), Ca\(^{2+}\), Fe\(^{3+}\), La\(^{3+}\), and Eu\(^{3+}\), resulted in precipitation of the AA-MWNT complex. The precipitates could be re-dispersed in water by chelating the metal cations with EDTA.

Slinski and coworkers also reported noncovalent functionalization of SWNTs with polysaccharides such as chitin, a natural polysaccharide consisting of the main chain but missing the side chains. Schizophyllum is a natural triple helix structure, which can be disassociated into single chain in DMF and can also be reconstituted by simply replacing DMF with water. The hollow helical column in schizophyllan is considered hydrophilic, similar to that in cyclodextrin, capable of entrapping hosting as-grown or shortened SWNTs. This was...
accomplished by mixing and sonicating schizophyllan with nanotubes in DMSO, and then exchanging with water. The results from microscopy analyses of these polysaccharide-SWNT host-guest complexes are quite interesting (Fig. 4).

3. Bioapplications

There has been much recent effort on demonstrating the feasibility and potentials for uses of carbon nanotubes in a variety of biological and biomedical systems and devices. Significant progress has been made to overcome some of the fundamental and technical barriers toward bioprocessing of carbon nanotubes, especially on issues concerning their aqueous solubility and their conjugation with or integration into bioactive molecules and/or biological species. Highlighted below are some interesting recent studies and achievements in representative areas of the broader field concerning bioprocessing of carbon nanotubes.

3.1. Biosensors

Carbon nanotubes, especially SWNTs, are size-wise comparable with many biomacromolecules (e.g., DNA on the order of 1 nm in size). The ultimate electrical properties and their sensitivity to changes in the surrounding environment have made carbon nanotubes ideal components in biosensors, such as electrodes for signal transmission and detectors for sensing chemical and biological materials. Both electrochemical sensors and those based on field effect transistor (FET) have been developed and/or fabricated in different configurations and mechanisms. A number of recent review articles on the subject, especially on electrochemical sensors, have been published.

Semiconducting SWNTs are widely considered as the most promising nanoscale molecular sensors for their extremely high sensitivity and fast response time. In general, the FET is constructed by a substrate (gate), two macroelectrodes (source and drain), and a SWNT (or SWNT network) bridging the electrodes. A SWNT FET is usually fabricated by casting a dispersion of bulk SWNTs or by directly growing the nanotubes via a chemical vapor deposition (CVD) method on a substrate either before or after the electrodes are patterned.

The functions of the transistors are associated with their diffusive electron transport properties. The current flow in SWNT FET is extremely sensitive to the substance adsorption or other related events, on which the sensing is based. For example, upon the binding of biological macromolecules to the nanotube in the device, a change in the charge state perturbs the current flow in the nanotube, thus producing detectable signals for the sensing. A wide variety of applications for such a device have been explored, including especially the detection of proteins, antibody-antigen interactions, glucose, DNA and DNA hybridization, and single nucleotide polymorphism.

The detection limit for the sensing of proteins or protein-protein interactions has generally been in the range of 100 pm to 100 nm. In a recent study, however, Byeou and Choi modified the geometry of the SWNT FET device to improve the sensitivity to 1 pm for both nonspecific and specific protein bindings.

Figure 4. (top) The chemical structures of schizophyllan and curdlan. a) TEM image of a-grown-SWNTx/SPC composite, and b) and c) a magnified picture. d) The original image of c) was Fourier filtered to enhance the contrast of the composite. Reproduced with permission from [50]. Copyright 2005 American Chemical Society.

Alternatively, synthetic oligonucleotides (i.e., aptamers) have demonstrated advantages (stability, small size, etc.) in the specific detection of amino acids.
drugs, and proteins. In the FET sensors, aptamer-modified carbon nanotubes were used for the detection of immunoglobulin E (IgE). The modification was the immobilization of 5'-amino-modified aptamer onto the nanotube surface with the aid of the linker molecule 1-phenyl-3-pyrazolonic acid succinimidyl ester. There was a sharp decrease in the source-drain current in the presence of the targeted IgE, and the net current change increased with the IgE concentration. The detection limit for IgE was 200 pM. In a comparison between devices based on the aptamer-modified and monoclonal antibody-modified carbon nanotubes, the former exhibited a better performance in the detection of IgE under similar experimental conditions.

Star et al. fabricated carbon nanotube FETs as selective detectors for DNA immobilization and hybridization. They fabricated an immobilized synthetic oligonucleotide specifically recognized the target DNA sequence, including a 16-base single-nucleotide polymorphism discrimination in HFE gene (responsible for hereditary hemochromatosis). Upon the addition of divalent cation Mg²⁺ there was a significant increase in the extent and overall efficiency of DNA hybridization on nanotubes, increasing the sensitivity by three orders of magnitude to push the detection limit down to 1 pM. Gui et al. employed the same FET configuration in the fabrication of a device with two different metal contacts (Au and Cu) for electrical detection of DNA hybridization.

The sensing mechanism for nanotube-based FETs has been debated, though it is generally acknowledged that unlike silicon nanowire devices, SWNT devices are operated in terms of the Schottky barrier modulation effect[45,46] as well as the chemical gating effect[47-50]. The former dominates when the isoelectric point of the targeted protein is close to the nanotube's pH[51]. According to a recent report, experimentally the effect of protein adsorption on the relationship (I-V curve) between source-drain current and the liquid gate potential could be used as a tool to identify the sensing mechanism in the SWNT transistors.[52] It was found that the sensing was indeed due to a combination of Schottky barrier and electrostatic gating effects. While earlier reports suggested that the sensing region was limited to the nanoscale contacts,[53,54] these new results seem to indicate that strong electrostatic gating could also occur along the bulk of the SWNT channel. The new finding was consistent with another recent study of DNA immobilization on back-gated SWNT network.[55]

There is still much room for further development in carbon nanotube FETs for biosensing, especially with respect to localizing nanoscale contacts of SWNTs with bio-surfaces and improving the fabrication of devices with complex arrays of semiconducting SWNTs. As discussed earlier, semiconducting SWNTs are used in FETs with care exceptions, however, as-produced SWNTs are mixtures of semiconducting and metallic nanotubes (in a ratio close to the statistical limit of 1:3-1:5). Therefore, selective removal of metallic SWNT for pure semiconducting ones, such as impurities and fabricated electrical breakdown down technique or taking advantage of the recent advances in post-production separation of semiconducting SWNTs from the as-produced mixtures, may prove highly beneficial. Finally, much remains to be explored on directly connecting living cells to these nanoelectronic devices for probing and understanding electronic responses in living systems.

3.2. Bio-Delivery

As an alternative to the extensively investigated drug delivery systems for improved pharmacological profiles and therapeutic properties, such as liposomes, polymers, dendrimers, and a variety of nanomaterials, carbon nanotubes have been praised for their potentially high loading capacity and the ability to penetrate into cells without the need for any external transporter system. There has been significant recent progress in the demonstration on using carbon nanotubes as effective carriers for shuttling and delivering various peptides, proteins, nucleic acids, and small molecular drugs into living cells.[56] Proteins were immobilized as "double functionalization" strategy to attach both fluorescein isothiocyanate (FITC, a fluorescent probe) and methotrexate (MTX, an anticancer drug) onto the sidewall of MWNTs via the 1,3-cycloaddition reaction of azomethine ylides.[57] Synthetically, the nanotubes were first co-functionalized with two orthogonal protected amino groups, followed by selective de-protection and then derivatization with FITC and MTX. According to in vitro experiments with Jurkat cells, the nanotube-bound drugs were rapidly internalized into the cells to accumulate in the cell cytoplasm.[58] The same group reported that phorboxizyn-copolymer-dispersed MWNTs could form supramolecular complexes with doxorubicin (DOX, a popular anticancer drug) via π-stacking of the DOX aromatic hydroxy-anthaquinonic rings on the nanotube surface.[59] The fluorescence intensity of the nanotube-bound DOX was suppressed due to the known quenching effect. According to the toxicological assay with MCF-7 human breast cancer cells, there was a significant enhancement in cytotoxic activity with the DOX-nanotube complex (the copolymer-DOX and free DOX as controls, and no cytotoxicity with the phorboxizyn-copolymer-dispersed MWNTs), and the enhancement was attributed to a more effective delivery of DOX with the aid of MWNTs.[60]

Dai and coworkers combined SWNTs with a large amount of DOX, where the complexation was noncovalent attachment again via π-stacking.[60] Two aqueous soluble SWNT samples were used, phospholipid (PL)-SWNT from dispersion with the surfactant PL-PEG (~1200 PEG units) and PEG-SWNT from the covariant functionalization of oxidized SWNTs with the PEG (220 units). The DOX attachment to and release from the nanotubes were found to be dependent on pH and the diameter of SWNTs, generally lower pH and smaller diameter in favor of DOX dissociation from the nanotubes. According to the in vitro toxicity experiments, the DOX-loaded PL-SWNT induced significant U87 cancer cell death and cell apoptosis (PL-SWNT without DOX as negative control), similar to free DOX, though the IC₅₀ (half-maximum inhibitory concentration) value for the nanotube-bound DOX (~8 μM) was higher than that of free DOX (~2 μM).[60] For specifically targeting U87 cancer cells, a cyclic arginine-glycine-aspartic acid (RGD) peptide (recognizing integrin α₅β₁ receptors up-regulated in solid tumors) was conjugated to the terminal groups in PL-SWNT. The effectiveness of the targeted delivery of the nanotube-bound DOX was reflected in the lower IC₅₀ value (~3 μM) for the RGD positive U87 cancer cells, with RGD-MWNT cells (short of corresponding receptors) as negative control showing no improvement in the IC₅₀ value (Fig. 5).
transcriptase (mTERT) expression to form the mTERT-miRNA/ SWNT complex. These specifically biofunctionalized SWNTs successfully entered three cultured mouse tumor cell lines, silenced the expression of the targeted gene, inhibited cell proliferation and promoted cell senescence in vitro, and also suppressed tumor in vivo. The functionalized SWNTs ready for the specific conjugation might represent a new class of molecular transporters for applications in gene therapeutics. Similarly, by being grafted onto carbon nanotubes, siRNA was also delivered into human T cells and primary cells, exhibiting superior silencing effects over conventional liposome-based nanoviral agents.

For eventual in vivo applications of the widely pursued carbon nanotube delivery systems, a critical challenge is to be able to keep the nanotubes in the bloodstream long enough for their intended functions. In a recent study by Wang, Sun, and coworkers, the functionalization of SWNTs with oligo-meric polyethylene glycol (PEGylation) was found to be remarkably effective in achieving prolonged blood circulation half-time on the order of 20 hours for the PEGylated nanotubes. Experimentally, skeletal 14C-enriched SWNTs were functionalized with the oligomeric PEGs by targeting the nanotube surface defects in well-established reactions. The plasma pharmacokinetic study was performed by injecting male KM mice intravenously with a solution of the PEGylated SWNTs, and by quantifying the nanotube concentrations in the blood post-exposure at different time intervals in terms of the 14C isotopic abundance determination (isotope ratio mass spectrometry). At one day post-exposure, for example, about 30% injected dose (% ID) remained in blood circulation, compared to only 0.2% ID for pristine SWNTs at the same time post-exposure. In a similar study by Dai and coworkers, a comparable blood circulation time was obtained for SWNTs noncovalently functionalized with branched PEGs. These were also preliminary results suggesting that the tumor uptake of the PEGylated SWNTs benefited from their prolonged blood circulation. For the EMF6 model (breast cancer in BALB/c mice) and the Lewis model (lung cancer in C57BL/6 mice), the tumor uptakes of intravenously administrated PEGylated SWNTs were 8% ID/g and 9% ID/g, respectively, considerably higher than those for SWNTs without covalent PEGylation (in the absence of any specific targeting moieties).

Mechanistically, the pathway of the carbon nanotube-enabled or associated delivery is still being debated, despite the experimental demonstrations of the efficiency of such delivery. One issue is on the entry mechanism that regulates the cellular
internalization of SWNTs and their carried cargos. Dai and coworkers proposed an endocytosis uptake mechanism on the basis of the observed temperature dependence in cellular uptake of carbon nanotubes, whereas the group of Kostarelos, Bianco, and Prato suggested an energy-independent non-endocytic mechanism involving insertion and diffusion of nanotubes through the lipid bilayer of the cell membrane. Experimentally, Dai and coworkers functionalized shortened SWNTs (30–200 nm in length) with DNAs and proteins noncovalently to study their uptake by HeLa (adherent) and HU30 (nonadherent) cells. According to their results, these short SWNTs transported the bioactive cargos into living cells in an energy-dependent fashion. They suggested that the endocytosis pathway for these well-dispersed, short SWNTs with biocompatible pips rather than cavioloe or lipid rafts. The same group also studied large aggregates of DNA-functionalized SWNTs (200 nm–2 μm long and up to 15 nm in diameter) for their uptake by HeLa cells. The results were similar to those of the shortened SWNTs, consistent with endocytosis. However, the mechanistic elucidation on the cellular uptake of these relatively large nanotube aggregates could be less certain due to complications such as their low solubility.

The group of Kostarelos, Bianco, and Prato disagreed by suggesting that the large biomolecules solubilizing carbon nanotubes could alter the nanotube interactions with cells and affect the intracellular transport kinetics. In experiments designed to support their suggestion and to examine key steps in the cellular uptake process, Kostarelos et al. functionalized both SWNTs and MWNTs with a wide range of molecules and bioactive species, including ammonium, small molecule fluorescent probes, anticancer drugs, and antibiotics. All of these functionalized nanotubes were found to be taken up by a variety of cells in vitro and subsequently trafficked through different cellular barriers to the perinuclear region, even under endocytosis-inhibiting conditions. The molecular simulation results also seemed consistent with the hypothesis that carbon nanotubes act as “nanoneedles” to pierce or penetrate the plasma membrane.

It appears that some of the disagreements or seemingly contradicting results on the cellular uptake mechanism might be due to different characteristics associated with the specific types of carbon nanotubes and/or different experimental procedures. More specifically, the non-endocytic internalization was found largely for small molecules covalently attached carbon nanotubes, while translocation via endocytosis was for larger biopolymers or aqueous soluble macromolecules conjugated SWNTs. Nevertheless, mechanistic debate is healthy and stimulating, and an improved mechanistic understanding is critical to further development of carbon nanotube transporters for delivery applications in vitro and in vivo.

There has also been a kind of delivery that is independent of any cellular uptake mechanism. Chen et al. developed a nanotube-based delivery configuration dubbed “nano-injector”, which went through the cell membrane via physical insertion. For the delivery of fluorescent quantum dots (QDs), biotinylated (via a disulfide linker) cytochrome c was synthesized for attachment (possibly via stacking) to MWNT as an atomic force microscopy (AFM) tip. The biotin moieties on the tip were readily conjugated with streptavidin-coated QDs (5–50 per nanotube). The nano-injection by the MWNT could deliver the QDs into a specific cell, which could then be released via the cleavage of the disulfide linker. A major advantage was suggested for this approach that the delivery/release processes could be repeated many times without cell damage.

3.3. Bioimaging

Potential cellular and sub-cellular imaging applications of carbon nanotubes (SWNTs in particular) have attracted much attention. These applications would take advantage of the unique physical and chemical properties of carbon nanotubes and also their exceptional capability in penetrating cell membranes. The feasibility of the bioimaging studies with carbon nanotubes have been in two categories: the direct optical imaging relying on the intrinsic fluorescence (including the band-gap emission of semiconducting SWNTs in the near-IR region and the visible emission due to passivated surface defects in functionalized SWNTs and MWNTs), and the indirect imaging based on nanotube-attached fluorescent or radioactive agents.

The band-gap fluorescence in individual semiconducting SWNTs was discovered by the group led by Weisman and Smalley. In as-produced SWNTs, typically about two-thirds are semiconducting. When these nanotubes were individualized in the solution involving no chemical modification or damage to the nanotubes, fluorescence emission could be observed in the near-IR spectral region. For semiconducting SWNTs of a nanometer in diameter and several hundred nanometers in length, the emission covers the spectral region of 900–1600 nm. Since most natural biomolecules are relatively transparent and nonresistive in this region, the sharp nanotube fluorescence spectra may be detected even in a more complex biological environment. The band-gap fluorescence emission is apparently sensitive to surface defects on the nanotubes, quenched in oxidized or functionalized SWNTs. However, as first reported by Sun and coworkers, both SWNTs and MWNTs with surface defects exhibit relatively strong photoluminescence upon chemical functionalization at the defect sites, and the emission is brighter with better functionalization. The defect-derived photoluminescence is excitation wavelength dependent in the visible and extending into the near-IR. Therefore, the well-functionalized carbon nanotubes are also amenable to optical bioimaging applications.

Dai and coworkers used semiconducting SWNTs as near-IR fluorescent tags for selective probing of cell surface receptors and cell imaging. The nanotubes were dispersed noncovalently with the amine-terminated surfactant PL-PEG-NH₂, and the resulting nanotube-bound residual amino groups were conjugated with thioketal Atto488 (an antibody recognizing the CD20 cell surface receptor) and Herceptin (recognizing the HER2/neu receptor on certain breast cancer cells). In solution, emissions of these antibody-conjugated SWNTs were in the 1000–1600 nm spectral region (at 780 nm excitation), namely that the known near-IR fluorescence of semiconducting SWNTs was preserved after the antibody conjugation. The fluorescence quantum yield was relatively low, but sufficient for the imaging experiments.
According to near-IR fluorescence imaging in vitro, there was specific binding of the antibody-conjugated SWNTs to the host cells. The fluorescence intensity results suggested high specificity for the different antibodies (551 and 201 for host carcinoma cells).

Cherel et al. used near-IR fluorescence to study the uptake of fluoronic surfactant-dispersed pristine SWNTs into macrophage-like cells. Macrophage samples that were incubated in growth media containing the dispersed SWNTs exhibited characteristic nanotube fluorescence spectra. The fluorescence intensities increased smoothly with incubation time and extracellular nanotube concentration. The near-IR fluorescence microscopy at wavelengths beyond 1000 nm yielded high contrast images showing the localization of nanotubes in numerous intracellular vesicles. It appeared that the cellular uptake of SWNTs was through phagocytosis. The population growth in macrophage cultures was unaffected by exposure to nanotubes at $\leq 4 \mu$g mL$^{-1}$ concentration. Lee et al. also used near-IR fluorescence microscopy to image SWNTs in organisms and biological tissues of nanotube-fed Drosophila larvae. The estimate was that only a very small fraction ($\approx 10$ ppb) of the ingested nanotubes became incorporated into organs of the larvae. These studies demonstrated that near-IR fluorescent SWNTs could be used as effective probes for potential diagnostic applications.

The much brighter visible (extending to the near-UV) photoluminescence in functionalized carbon nanotubes has also found applications in bioimaging. Lucchini et al. used SWNTs that were covalently linked with $\text{NH}_3$-terminated aliphatic amines (SWNT-NH$_2$), emission peak at 483 nm with 995 nm excitation in confocal laser scanning microscopy to visualize the interaction of SWNT-NH$_2$ with human carcinoma lung carcinoma A549 cells (Fig. 6).

There was the intracellular and perinuclear localization of SWNT-NH$_2$, but no cell plasma membrane damage at a dose up to 500 $\mu$g mL$^{-1}$ and 24 h post-incubation. This was the first report on the visible fluorescence imaging of SWNTs in cells without the need for large fluorescent labels attached to biopolymers or macromolecules. The imaging methodology thus developed may serve as a widely applicable tool for elucidating the intracellular transport mechanism of carbon nanotubes.

For indirect optical imaging, carbon nanotubes essentially serve as carriers for the fluorescent molecular labels. In the study by Dai and coworkers, as-produced SWNTs were sonicated in an aqueous solution of fluorescein-poly(ethylene glycol) (Fluor-PEG, 114 PEG units) and purified via centrifugation to obtain Fluor-PEG-functionalized SWNTs. Compared to those of free fluorescein, the absorption peak of the nanotube-attached Fluor-PEG in phosphate buffer solution (pH 7.4) was red-shifted by several nanometers, and the fluorescence was quenched by about two-thirds. According to the bioevaluation results, these functionalized SWNTs served as both intracellular transporter and fluorescent marker, with their uptake by mammalian cells (B1474 breast cancer cells) and also their enabling the detection of fluorescence inside the cells.

In addition to fluorescence-based imaging, radiotracers tracing and the use of special microscopy techniques have also been effective in the study of carbon nanotubes in vitro and in vivo. For example, Porter et al. visualized individual SWNTs in cells through a new technique called low-loss energy-filtered transmission electron microscopy in combination with electron energy loss spectrum imaging. The technique made it possible to directly determine the distribution of SWNTs in both stained and unstained cells. It also enabled the tracking of cellular actions of the nanotubes, such as their entering the cytoplasm, localizing...
3.4. In vivo Biodistribution and Toxicity

The widespread interests and rapid advances in the use of carbon nanotubes for new materials and technologies have promoted growing bio-safety concerns and the recognition that a fundamental understanding of the pharmacological and toxicological properties of carbon nanotubes is necessary to even urgent. As a result, there have been increasing activities in the respective areas.\(^{12,13}\)

Biocompatibility and biodegradability of carbon nanotubes in vivo are important to many of the currently pursued biosensory applications. A number of studies have been commissioned with aims toward systematic and quantitative in vivo analyses of carbon nanotubes on issues such as distribution, metabolism, degradability, clearance, and biodistribution. For pristine carbon nanotubes (not chemically modified or functionalized) in biological systems, there have been more experimental challenges due to a general lack of quantitative detection methods. Yang et al.\(^{16}\) developed an approach to use skeletal \(^{13}\)C-enriched pristine SWNTs for quantification with isotope ratio mass spectrometry. The biodistribution of the SWNTs (\(^{13}\)C-enriched) in mice was determined at different time intervals post-exposure.\(^{16}\) As shown in Figure 7, the SWNTs were apparently cleared from blood stream quickly and distributed throughout most organs within 24 h. The primary accumulations were in lungs, liver, and spleen, and the nanotubes were retained in these organs at relatively high accumulation levels over 28 days. Most noticeable and interesting was the possible accumulation (1-3% ID) in the brain (for the SWNTs to cross the blood brain barrier).

While the accumulation level of SWNTs in liver was relatively constant, there was a gradual decrease of accumulation in lungs from 15% ID to 9.4% ID in the monitoring period, for which secretion by the alveolar macrophage as a result of mucociliary transport and translocation through the lymph nodes would be considered as possible clearance pathways. The pristine nanotubes could hardly be detected in urine and feces in terms of either \(^{13}\)C isotope ratio mass spectrometry measurements or TEM analyses, obviously different from their modified or functionalized counterparts (reported to be cleared from the animal mostly through the renal excretion route).\(^{13,19}\)

For functionalized carbon nanotubes, radiotracers have become a generally adoptable method for visualizing their biological behavior in animals, with \(^{11}\)C, \(^{12}\)C, \(^{13}\)C, \(^{14}\)C, \(^{51}\)Cr, and \(^{59}\)Fe as radiolabels. In the study by Singh et al.\(^{21}\) sidewall functionalized SWNTs including the radiolabel \(^{11}\)C was intravenously administrated into mice. The nanotubes were rapidly cleared from systemic blood circulation (half life ~3 h) through the renal excretion route, without being retained in any of reticuloendothelial system (RES) organs.\(^{21}\) For comparison, the same group prepared three SWNT samples, including purified MWNTs with serum protein coating and the diethylaminoethylamino dithioletriazine (DETA) functionalized MWNTs with and without the radioactive \(^{11}\)C label, for the administration into mice intravenously.\(^{20}\) The purified MWNTs were accumulated in liver and lungs, but no significant accumulation for the DETA-functionalized MWNTs. In another study by Chen, Dai, and coworkers,\(^{22}\) SWNTs from the HPLC purification technique were wrapped in the surfactant PL-PEG with the radiolytic \(^{13}\)C label for the investigation on biodistribution and tumor targeting ability in mice by using in vivo positron emission tomography (PET), ex vivo biodistribution analysis, and Raman spectroscopy. It was found that the SWNTs wrapped with PL-PEG were surprisingly stable in vivo and exhibited relatively long blood circulation times and low RES uptake. These nanotubes upon linking to an RGD peptide could also efficiently target integrin-positive tumor in mice.\(^{23}\)

As demonstrated in the studies highlighted above and others, radiolabeling of the organic molecules on nanotube surface can be readily achieved. However, the labeled nanotube conjugates may suffer from decreasing or even losing activity over time due to decay or dissociation (from the nanotubes) of the label. Thus, direct detection and quantification of carbon nanotubes in vivo and/or ex vivo based on the intrinsic physical and chemical properties of the nanotubes could be more advantageous in some studies to monitor the biological behavior of carbon nanotubes over both short and long terms. For example, Liu et al. used intrinsic Raman signature to probe the blood circulation and long-term fate of SWNTs noncovalently functionalized with several linear and branched PL-PEGs in mice.\(^{24}\) The coating with PEGs was very effective to achieve long blood circulation and low RES uptake, with the near-complete clearance from main organs via biliary and renal pathways occurring in about 2 months\(^{25}\) (probably due to improved hydrophilicity and resistance to protein nonspecific binding). Cherukuri et al. used near-IR fluorescence of individualized SWNTs to determine the blood elimination kinetics and biodistribution of the nanotubes in rabbits (Fig. 8).\(^{26}\) It was found that the nanotube concentration in the blood serum decreased exponentially with a half-life of one hour, and significant nanotube concentration was detected only in the liver at 24 h after intravenous administration.\(^{26}\) Yang et al. again applied isotope ratio mass spectrometry, coupled with the covalent functionalization of skeleton \(^{13}\)C-enriched SWNTs with the diamine-terminated PEGs (PEGylation), to the determination of in vivo biodistribution.\(^{27}\) These PEGylated SWNTs intravenously administrated into mice were distributed throughout most organs within one hour and...
Figure 8. Micrographs at two magnifications of liver tissue from rabbits killed 24 h after i.v. administration of suspended SWNTs. A) and B) Near-IR SWNT fluorescence images with field-of-views of 390 μm (A) and 83 μm (B). Scattered isolated bright pixels are artifacts from defective sensor elements in the near-IR camera; all larger features represent emission from SWNTs. In C) and D), the SWNT fluorescence from A and B is shown overlaid as false-color green onto visible bright-field images from adjacent 3-μm-thick specimen slices that had been stained with hematoxylin and eosin. Reproduced with permission from [106]. Copyright 2006 National Academy of Sciences, USA.

significantly accumulated in liver and spleen, similar to pristine SWNTs. At day 7 post exposure, ~25% ID and ~3% ID of the PEI-gated SWNTs remained in liver and spleen, respectively. However, their uptake by RES was significantly reduced (28% ID) in comparison with that of the corresponding pristine SWNTs (37% ID). According to this and other studies, there are obvious differences between pristine and functionalized SWNTs in their biodistributions and time dependencies post-exposure, suggesting that the surface functionality and morphological and dispersion characteristics of carbon nanotubes do have significant biological consequences.

It has made in headlines that carbon nanotubes might potentially be toxic. Carbon nanotubes may enter the body via many routes, such as intravenous, dermal, subcutaneous, inhalational, intraperitoneal, or oral. Preliminary in vivo toxicity studies on respiratory and skin exposure to pristine carbon nanotubes did show some harmful effects, so that there should be precautions to limit the exposure. However, the toxicity of carbon nanotubes seems to depend on many factors, including dosage, physical form, and chemical attachment.

In an in vitro study, Cui et al. explored the biocompatibility of as-produced SWNTs with the human HEK293 kidney cells. They found that the nanotubes could inhibit cell proliferation and at the same time decrease the cells' ability to adhere in a dose and time-dependent manner. In another recent study cited at issues concerning the lack of aqueous solubility and the contamination of residual metal catalysts in carbon nanotubes, Izis-Borgman et al. used water-soluble and metal-free carbon nanotube aggregate (covalently bonded class of self-aggregated nanotubes) to examine cytotoxicity on 3T3 and HeLa cell lines. The results suggested very low cytotoxicity, even lower than that of quartz microparticles, though there were still doubts on the reliability of the results because of some experimental problems. The structural differences between nanotubes and nanotubes should also be noted.

Chemically modified or functionalized carbon nanotubes and thus aqueous soluble have generally exhibited no apparent or less cytotoxicity to all living cell lines that have been investigated.

For example, Demir et al. found that carbon nanotubes functionalized in 1,3-dipolar cycloaddition reaction or treated with oxidation and then amidation could be taken up rapidly by B and T lymphocytes as well as macrophages without affecting the overall cell viability. It was also found that the highly water-soluble modified carbon nanotubes did not affect the functional activity of different types of immune-regulatory cells.

In the in vivo experiments on pristine and the PEI-gated SWNTs in mice, Yang et al. did not find any of the animals exhibiting any signs of acute toxicity response during the experimental period, even at a high exposure of 30 mg pristine or 2 mg PEI-gated SWNTs (nanotube equivalent, both skeleton 13C-enriched) per kilogram body weight, consistent with other reports. In a recent toxicity study by Schipper et al., the covalently and noncovalently functionalized SWNTs were injected into a small number of mice, and results suggested no evidence of toxicity over 4 months.

In fact, overall the available in vivo biodistribution studies have generally reported no acute toxicity or negative health effects of the carbon nanotubes on the animals involved in the experiments. However, further investigations are necessary and are necessary in progressing in many laboratories on the potential toxicity and related issues.

4. Summary

The research on the modification and functionalization of carbon nanotubes has become an increasingly mature topic. As a result, many biocompatible and/or bioactive carbon nanotubes and related systems have been developed and synthesized for widespread investigations on their potential biomedical applications. Since our last report, there has obviously been a dramatic proliferation of activities in this field. The positive trend and also the expansion into other areas that are not covered in this report are expected to continue in a rapid pace. In further studies, however, more efforts should be and will likely be devoted to demonstrate that carbon nanotubes not only "can be used" but also "are uniquely suited" for various applications in biology and medicine.

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Carbon Nanosheets for Polymeric Nanocomposites with High Thermal Conductivity

By L. Monica Veca, Mohammed J. Meziani, Wei Wang, Xin Wang, Fushen Lu, Puyu Zhang, Yi Lin, Robert Fee, John W. Connell, and Ya-Ping Sun

Carbon materials are known for their thermal-conductive properties. Recently, carbon nanotubes (with an experimentally measured individual-nanotube thermal conductivity of 30000 W m⁻¹ K⁻¹) and a value of up to 6600 W m⁻¹ K⁻¹ predicted theoretically³⁶) have generated much excitement because of their potential use in polymeric nanocomposites with ultrahigh thermal conductivities.⁴⁻¹¹ So far, however, no such nanocomposites have been produced. Alternatively, there is increasing attention being paid to the use of exfoliated graphite in polymers to produce thermally conductive nanocomposites.¹²⁻²¹ since the thermal conductivity of graphene was estimated to be as high as 5300 W m⁻¹ K⁻¹.¹²⁻¹³ For example, Doral and co-workers¹¹ reported that graphite could be intercalated via chemical oxidation, then rapidly exfoliated at a high temperature; the exfoliated graphite could then be dispersed into polymeric matrices, including nylon and polyethylene, to produce composites with enhanced thermal conductivities. It was found that the thermal conductivity of the composites increased almost linearly with the graphite loading, with a value of up to 4.1 W m⁻¹ K⁻¹ being recorded for a composite of nylon-6 with 30 vol% exfoliated graphite (compared to only 0.25 W m⁻¹ K⁻¹ for the blank polymer).¹² Similarly, Holden and coworkers processed natural graphite flakes into “graphite nanoplatelets” (GNPs) by first treating them with a mixture of concentrated sulfuric acid and nitric acid for intercalation and then exfoliating via thermal shock upon rapid exposure of the intercalated graphite to various high temperatures in nitrogen.¹⁷ The GNPs thus obtained were dispersed via a post-processing treatment, in order to fabricate composites with epoxy. These GNP-epoxy composites were found to have thermal conductivities up to 6.44 W m⁻¹ K⁻¹ at 25 vol% GNP loading, which is considerably higher than the value of the blank epoxy.¹⁹

Connell and coworkers²⁰ compared the thermal conductivities of extended composite ribbons made of different nanoscale carbon fillers, including multilayered carbon nanotubes (MWCNTs), vapor-grown nanofibers, and commercially available expanded graphite, in Ultem 1000 resin. The expanded graphite was found to be most effective, with the in-plane thermal conductivity reaching 6.7 W m⁻¹ K⁻¹ in the ribbon sample containing 40 wt% graphite.

In this work, it will be reported that commercially available expanded graphite could be further exfoliated in a specifically designed process to produce nanosheet-sized carbon structures (dubbed “carbon nanosheets”). These carbon-nanosheet-based materials were used as fillers in polymeric nanocomposites that exhibited ultrahigh thermal conductivities, setting, to the best of our knowledge, a new record.

The processing required to produce the carbon nanosheets involved sequential alcohol and oxidative acid treatments. First, the as-supplied expanded-graphite sample was added to an alcohol/water mixture.²² The resulting suspension was stirred at room temperature and then vigorously sonicated. The graphite sample recovered post-treatment was added to a nitric acid/sulfuric acid mixture that was preceded in an ice bath. Upon vigorous sonication, the processed graphite sample was recovered by rather straightforward procedures, including filtration, washing with water until the pH value was neutral, and drying in a vacuum oven.

The combination of alcohol and oxidative acid treatments yielded samples with a substantial presence of carbon nanosheets, according to characterization by microscopy and other techniques. A typical transmission electron microscopy (TEM) image of the processed sample is shown in Figure 1. The edges of the carbon nanosheets, which tend to scroll and fold, as seen in the image, were used to estimate the thickness of the nanosheets, which was generally on the order of 2–8 nm. The electron-diffraction pattern shown in Figure 1 corresponds to two dominant periodicities of 2.13 and 1.33 Å, similar to those reported in the literature for thin crystalline graphene sheets.²³

Also shown in Figure 1 is a typical atomic force microscopy (AFM) image of the carbon nanosheets obtained from the processed sample on a mica surface, where the height profile is consistent with the thickness of the nanosheets estimated in the TEM analysis (sheet dimension generally on the order of a micrometer).

With regards to bulk properties of the sample, powder X-ray diffraction results obtained before and after the alcohol and oxidative acid treatments showed a slight increase in the interlayer distance and, more meaningfully, a significant broadening in the diffraction peaks of the post-treatment sample. According to calculations based on the peak broadening using the Scherrer equation,²⁴ the average thickness of the graphite layer...
decreased from more than 20 nm in the pretreatment sample to 6–7 nm in the post-treatment sample, again in reasonable agreement with the TEM results.

The Raman spectroscopy results for the same samples were also consistent with a substantial presence of carbon nanosheets. The observed Raman G-band shifted to higher frequencies, indicative of a decrease in the number of graphene layers. According to a demonstration by Elsund and coworkers, the decrease in the number of graphene layers (from 19 to 1 in their work) is correlated with the progressive shift of the frequency of the observed Raman G-band.

To fabricate the polymeric nanocomposites, a weighed amount of the processed-graphite sample (carbon nanosheets) was suspended in dimethylformamide (DMF) via sonication, and the suspension was added dropwise to a solution of epoxy polymer (weight-average molecular weight, Mw ~ 26 000) in hot DMF with stirring. The resulting mixture was concentrated, vigorously stirred, and subsequently cast onto an iced glass slide. The slide was purged with nitrogen gas and then immersed in water for 30 min to obtain a freestanding nanocomposite thin film (around 50 nm thick). After careful drying, the film was used in thermal-diffusivity measurements.

The in-plane thermal diffusivity of the freestanding film was determined at room temperature using an instrument based on the laser-heating Angetrom method. At a carbon-filler content of 33 vol% in the epoxy nanocomposite thin films (from repeated fabrication under the same conditions), the experimentally measured in-plane thermal diffusivity averaged 33 mm²/s (values higher than 46 mm²/s were recorded for other samples), which is several orders of magnitude higher than the value of the blank epoxy polymer film (0.12 mm²/s). The thermal conductivity (λ) was calculated from the thermal diffusivity (D) using the equation

$$\lambda = \rho C_p D$$  \hspace{1cm} (1)

where ρ (density) and Cp (specific heat) of the nanocomposite were estimated from the respective values of the polymer and the filler using the commonly used mixing rule. Again for the epoxy nanocomposites with 33 vol% carbon nanosheets, the λ values thus calculated (close to 80 W m⁻¹ K⁻¹ on average) are much higher than those ever reported in the literature for composites of epoxy (or any other polymer) with exfoliated graphite fillers. For example, the previously reported high in-plane thermal conductivity in composites of the Ultem 1000 resin with expanded graphite was less than 7 W m⁻¹ K⁻¹ at comparable graphite loadings.

The carbon nanosheets were used with other polymeric matrices to fabricate nanocomposite thin films. The thermal diffusivities of these films varied somewhat from polymer to polymer, but remained high. For example, with polynorbornene (obtained from the condensation of 4,4'-dihydroxydianorbornenediyl) and 1,3-bis(3-aminophenoxynyl)benzene, often referred to as 1,4(1H,4H)-1,4-poly[9,9-doped polymeric acetylene (I)], the nanocomposite thin films exhibited in-plane thermal diffusivities on the order of 20 mm²/s at carbon-nanosheet loadings similar to those used in the epoxy nanocomposites discussed above. While less spectacular (compared with the average 33 mm²/s in the epoxy nanocomposites), these thermal-diffusivity values still set records for their respective polymeric nanocomposites.

As expected, the thermal diffusivities of the polymeric nanocomposites are strongly dependent on the carbon-nanosheet content, with higher volume fractions consistently resulting in higher thermal diffusivities of the nanocomposites. This is demonstrated in Figure 2, which shows the dependence of the thermal diffusivity on the volume fraction of nanosheets in the epoxy-carbon-nanosheet thin films.

The thermal-conductive properties of the polymeric nanocomposites produced with carbon nanosheets are highly anisotropic, with a large ratio between the in-plane and cross-plane thermal conductivities. For example, in thin films of the epoxy-polymer nanocomposite with a 33 vol% loading of carbon nanosheets, the experimentally measured cross-plane thermal diffusivities were generally about one-tenth to one-fifth of the average in-plane value discussed above. The highly anisotropic nature in the thermal conductivities of the nanocomposites is interesting but hardly surprising, and is likely a reflection of the pseudo-2D structure of the carbon nanosheets and their associated 2D thermal-conductive properties. Thus, these polymeric composites of carbon nanosheets should be particularly useful in potential applications that require efficient directional thermal transport.
Figure 2. Dependence of the observed thermal diffusivity on the carbon nanosheet loading in the epoxy-nanocomposite thin films.

Results from powder X-ray diffraction measurements of the carbon nanosheets were generally unchanged after dispersion of the nanosheets in the polymeric matrices. The dispersed carbon nanosheets were examined using cross-sectional TEM imaging. A thin film was microtomed in the direction perpendicular to the film surface, to yield slices less than 100 nm thick (used as specimen for high-resolution TEM imaging). Shown in Figure 3 are representative TEM images of the specimen at various resolutions, which essentially correspond to a direct view (from a cross-sectional orientation) of the nanoscale structure in the nanocomposite thin film that was microtomed. These images confirm that the graphitic fillers dispersed in the polymer matrix are indeed carbon sheets of nanoscale thickness (generally less than 10 nm).

The polymeric nanocomposites with carbon nanosheets appeared black in color, and remained elastic even at relatively high carbon loadings. As an example, the photographs in Figure 4 demonstrate the high degree of flexibility of the epoxy nanocomposite thin film with a 35 vol% carbon-nanosheet loading. Polymeric nanocomposites with lower carbon-nanosheet loadings were even more elastic and processable (they were melt-extruded into fiberlike structures and flexible tubing, for example). However, “films” of neat carbon nanosheets (without any polymer) were morphologically quite different, as expected. The preparation, appearance, and physical characteristics of these films were essentially the same as those of “bucky paper” made of single-walled carbon nanotubes, but the thermal conductivities were significantly higher (Fig. 2). At comparable loading levels, the carbon nanosheets were apparently more effective fillers than carbon nanotubes (both single-walled and multiwalled) for producing polymeric nanocomposites with high thermal conductivity. It has been acknowledged in the literature that the thermal conductivity of polymeric nanocomposites is mechanistically limited by the polymer/nanofiller interfacial thermal resistance. Heat transport in polymeric nanocomposites is carried out by phonons of varying frequencies, with the phonons slowing down at the polymer/nanofiller interface as a result of material characteristics, such as the largely amorphous nature of the polymer. However, it is difficult to hypothesize any fundamental difference in the polymer/carbon interfaces between the carbon nanotubes and
nanosheets (and thus possibly accounting for their significant difference in performance). Instead, it could be that the nanosheets, as 2D nanofilms, reduce the overall number of polymer/nanofiller interfaces for heat transport in the resulting polymeric nanocomposites. Further investigation of the polymeric nanocomposites composed of carbon nanosheets is needed for better structural and mechanical elucidation. Nevertheless, it is remarkable that relatively inexpensive graphitic materials could be used with various polymers and with relatively simple processing to produce nanocomposites of record-setting anisotropic thermal conductivities, which may prove highly valuable in many technological applications.

Experimental

Materials: the expanded graphite (surface-enhanced flake graphite, grade 3405) sample was supplied by Asbury Carbons. The bisphenol A epoxy-based polymer (EPON 828, resist 51-BH35, Mw = ca. 26000) in 2 solvent mixture of methyl ethyl ketone and propylene glycol monomethyl ether was obtained from Henkel Specialty Chemicals. The polymer was recovered by precipitating in water and then completely removing the water. Poly(vinyl alcohol) (PVA, Mw = ca. 70000-90000) was purchased from Aldrich, and LAB-CP a polyethylene (number-average molecular weight, Mn= ca. 17000, obtained from the condensation of 4,4’-bis(fluorenylcarboxyphényl)-1,2-dimethylpropane) from SIR. Inc. These polymers were used as received. Sulfuric acid (95%), nitric acid (71%), and ethanol were obtained from ACROS, and DMF from Mallinkrodt.

Carbon Nanosheets: the as-supplied expanded graphite sample was processed via a combination of alcohol and oxidative acid treatments. In a typical experiment, the sample (7 g) was added to an alcohol/water mixture (13:7 v/v, 400 mL), stirred at room temperature for 24 h, and then oxidized with 30% H2O2 (10 mL) for another 2 h. The sample was collected via filtration and then dried in a vacuum oven. A portion of this sample (500 mg) was added to a nitric acid/sulfuric acid mixture (13 v/v, 80 mL) that was proceed in an ice bath. After oxidation for 2-3 days, the mixture was transferred into water (1 L). Then, the processed graphite was collected via filtration, washed repeatedly with deionized water until a neutral pH was achieved, and dried in a vacuum oven.

Nanocomposite Films: an epoxy-polymer sample was dissolved in hot DMF (about 3 mg/mL). Separately, the carbon-nanosheet sample (50 mg) was suspended in DMF (10 mL) via sonication for 1 h. The suspension was added dropwise to the hot DMF solution of the epoxy polymer (10 mL) under stirring. The mixture was concentrated, stirred vigorously for 12 h, and then cast into thin films (30-80 μm thick) on etched glass slides in a glovebox under nitrogen. The slides were immersed in water for 30 min to allow the nanocomposite thin films to be released, and the freestanding films were subsequently dried in a vacuum oven (80-100°C) before characterization.

Similarly, the polyamide and PVA polymers were dissolved in DMF and hot water, respectively. Suspensions of the carbon nanosheets in DMF and water were prepared via homogenization (Omni International, THP115) for 30 min and then sonicated for 30 min. The suspensions were added dropwise to the polymer and PVA solutions, respectively, with stirring. The resulting mixtures were concentrated, stirred vigorously for 12 h, and then cast into thin films on glass slides in a glovebox under a nitrogen atmosphere. The freestanding films were dried in a vacuum oven (80-100°C) before measurements were taken.

Thin films of carbon nanosheets were only prepared by filtering the solutions of carbon nanosheets and then drying in a vacuum oven at 80-100°C.

Measurements: X-ray powder diffraction measurements were carried out using a Scintag XDS-2000 powder diffraction system. Raman spectra were measured using a Jobin Yvon T64000 Raman spectrometer equipped with a Melles-Griot 35 mW He-Ne laser source providing 633 nm excitation, a triple monochromator, an Olympus BX-41 microscope, and a cooled Symplyx detector. AFM images were obtained in the atomic force mode using a Molecular Imaging PicoPlus system equipped with a multipurpose scanner for a maximum imaging area of 10 μm x 10 μm and a Nanoworld Pointprobe NCH sensor (135 μm in length). The height-profile analysis was facilitated using the SPIP software distributed by Image Metrology. TEM was performed using a Philips CM-2000 S-TEM system and a Hitachi H-9500 TEM system; the selected-area diffraction measurements were performed on the latter. Carbon-coated or holey-carbon-coated copper grids were used in the imaging experiments. For cross-sectional imaging, a film sample was first embedded into epoxy resin and then microtomed into slices less than 100 μm thick using a Reichert jung Ultracut E Microtome with a 30°-angle diamond knife at room temperature.

The in-plane thermal diffusivity in the freestanding polymeric nanocomposite thin films was determined using an Ulvac LaserHot thermal diffusivity/conductivity meter operated at room temperature in a vacuum of 0.017 Pa. The thin films measured were about 100 μm x 4 mm in size, with one surface (facing the laser in the instrument) coated with a thin layer of graphite. At least three frequencies were used in the measurement of each film sample, and the readings were averaged for the given specimen. The cross-plane thermal diffusivities of the films were obtained using a Netzsch LSA 447 Nanoflash Instrument (Stony Flash lamp, 304 V, and long pulse) [20,13]. The density and specific heat of the nanocomposite were estimated from those of the polymer and filler with the commonly used mixing rule [27,23]. Composite = \( \rho_{\text{composite}} = \rho_{\text{polymer}} + \rho_{\text{filler}} \times \frac{\rho_{\text{filler}}}{\rho_{\text{polymer}}} \), where \( \rho \) denotes the respective weight fractions.

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Carbon Dots for Optical Imaging in Vivo

There has been significant recent interest in the development of highly fluorescent nanomaterials as contrast agents for optical imaging in vivo. The imaging agents should ideally be bright, nontoxic, biocompatible, and stable against photobleaching. Among those extensively studied are ones based on semiconductor quantum dots (QDs) such as CdSe/ZnS. The rationale for the use of QDs over conventional organic dyes is now generally accepted in the literature. There are already successful in vivo imaging demonstrations of QDs on tumor vasculature, tumor-specific membrane antigens, sentinel lymph nodes, and so on.7,4

Semiconductor QDs containing cadmium or other heavy metals are unfortunately known for their significant toxicity even at relatively low concentrations, which may prove prohibitive to any patient studies. Therefore, the search for benign alternatives has continued. Of particular interest and significance was the recent finding that small carbon nanoparticles could be surface-passivated by organic molecules to become strongly fluorescent.1 These fluorescent carbon nanoparticles,4 dubbed “carbon dots” (C-Dots, Scheme 1), were found to be physicochemically and photochemically stable and nontoxic. The carbon particle core could also be doped with an inorganic salt such as ZnS before the surface functionalization to significantly enhance the fluorescence brightness (C$_{\text{ZnS}}$-Dots, Scheme 1).1,7 These carbon dots have successfully been used for in vivo cell imaging with both one- and two-photon excitations.7,9,10

Carbon is hardly considered as an intrinsically toxic element. Available results from the ongoing toxicity evaluation of oligomeric PEG-functionalized C-Dots in mice have suggested no meaningful toxic effects, raising the prospect for in vivo biocompatibility and use of carbon dots. Here we report the first study of carbon dots for optical imaging in vivo. The results suggest that the carbon dots are not only brightly fluorescent in solution, as reported previously, but also well-behaved as contrast agents in live mice.

C-Dots and C$_{\text{ZnS}}$-Dots with PEG diamine, H$_2$N(CH$_2$)$_{12}$-CH$_2$NH$_2$ (where $\approx$ 55 PEG chains), as the surface passivation agent were prepared and characterized as previously reported.9,10 Shown in Figure 1 are representative atomic force microscopy (AFM) and high-resolution transmission electron microscopy (HRTEM) images of the carbon dots. Both samples were readily soluble in water to form stable aqueous solutions suitable for the various injections described below.

For subcutaneous injection, female DBA/1 mice (~25 g) were shaved in the back area surrounding the injection point. Upon injection of a C-Dots solution (30 μg carbon-core equivalent in 10 μL) or a C$_{\text{ZnS}}$-Dots solution (65 μg in 30 μL), the mice were imaged in a Lumina FL in vivo imaging system (MAG Biosystems) with 470 nm (~40 nm fwhm) excitation and 525 nm (~47 nm fwhm) emission filters. As shown in Figure 2, the fluorescence images of the subcutaneously injected mice exhibited bright emissions from C-Dots and C$_{\text{ZnS}}$-Dots. The relatively stronger fluorescence from the latter is consistent with the previously reported solution-phase results. The injected carbon dots in mice diffused relatively slowly, and the fluorescence faded at ~24 h postinjection.

The carbon dots could be excited at longer wavelengths for red fluorescence emission. For the same subcutaneous injection into mice, the imaging results with 545 nm (~29 nm fwhm) excitation and 620 nm (~59 nm) emission filters also exhibited significant fluorescence from both C-Dots and C$_{\text{ZnS}}$-Dots (Figure 2).

The brighter green fluorescence of C$_{\text{ZnS}}$-Dots was used in the imaging to track the migration through lymph vessels. Upon intraperitoneal injection into the front extremity (10 μg in 10 μL), the carbon dots migrated along the arm (Figure 3). Unlike semiconductor quantum dots, the carbon dots are noncristalline and show a high brightness with a small hydrodynamic diameter of ~4 nm.
dots such as CaSe/ZnS, which can migrate to axillary lymph nodes in minutes. The observed migration of the carbon dots was slower. This could be due to the small sizes of the carbon dots (on the order of 4–5 nm) and the surface functionalization by the FEGs, whose protein resistance characteristics might reduce interactions of the carbon dots with the lymph cells. The axillary lymph nodes were harvested and dissected at 24 h postinjection and exhibited readily detected fluorescence from the carbon dots (Figure 5).

A C-Dots solution (400 μg in 200 μL) was intravenously injected into mice for whole-body circulation. The abdomen was shaved for fluorescence detection of the dots trapped in organs during the circulation, but only emissions from the bladder area were observed (Figure 4). At ~3 h postinjection, bright fluorescence in the urine became visible in the imaging facility (Figure 4). The results suggest that the intravenously injected carbon dots are primarily excreted via urine, an excretion pathway that has been widely reported in the literature for FEGylated nanoparticles, especially for very small particles like the ones used here. The organs were harvested at 4 h postinjection for imaging analyses ex vivo. Only the dissected kidneys and liver exhibited meaningful fluorescence from the carbon dots, which was brighter in the former (Figure 4), consistent with the urine excretion pathway. The relatively weak fluorescence in the dissected liver was an indication of a low accumulation level of the carbon dots. While generally significant hepatic uptake of nanoparticles and nanocubes has been widely observed and discussed in many studies, the low accumulation here might again be attributed to the effective surface FEGylation that probably reduced the protein affinity and made the carbon dots stealthy with respect to hepatic uptake.

All of the reported animal experiments were performed at Chemos University by strictly following the IACUC-approved protocols. During the experiments, no animal exhibited any sign of acute toxicological responses.

In summary, the results reported here demonstrate that carbon dots injected in various ways into mice remain strongly fluorescent in vivo, which, coupled with their biocompatibility and nontoxic characteristics, might offer great potential for optical imaging and related biomedical applications.

Acknowledgment. We thank Hilary Hicks of MAG Biosystems for experimental assistance. This work was supported primarily by a Susan G. Komen for the Cure Postdoctoral Fellowship Award (L.C. and Y.-P.S.) and the NIH (Y.-F.S.). H.W. and Y.L. also acknowledge financial support from the NSFC and China Ministry of Science and Technology.

Supporting Information Available: Complete ref 7 and additional experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

References


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Figure 2. Subcutaneous injection of (top) C-Dots and (bottom) C-Dots: (a) bright field, (b) as-determined fluorescence Stokes–Stokes wavelengths indicated), and (c, e) color-coded images (Image from NIH).

Figure 3. Intraluminal injection of C-Dots: (a) bright field, (b) as-determined fluorescence, and (c) color-coded images. Insert: dissected in the intraluminal area axillary lymph nodes (LN).

Figure 4. Intravenous injection of C-Dots: (a) bright field, (b) as-determined fluorescence (BL, bladder; Ur, urine), and (c) color-coded images. The same order is used for the images of the dissected kidneys (a–c) and liver (a”–c”).

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Biodefunctionalization of Functionalized Single-Walled Carbon Nanotubes in Mice

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Chemically modified carbon nanotubes with hydrophilic functionalities such as polyethylene glycol (PEG) are widely pursued for potential biological and biomedical applications. In this study, PEGylated single-walled carbon nanotubes (PEG-SWNT) were intravenously administered into mice to study their biodefunctionalization in vivo by using complementary Raman and photoluminescence measurements. There was meaningful de-functionalization of PEG-SWNT in liver over time, but not in spleen under similar conditions. The evidence from spectroscopic characterization and analyses is presented, and mechanistic implications are discussed.

Introduction

Chemical modification and functionalization of single-walled carbon nanotubes (SWNTs) are generally considered as necessary for many of their widely pursued potential biomedical applications. For example, the functionalization of SWNTs helped to improve their pharmacokinetics and reduce toxicity. In particular, PEGylation (the functionalization with polyethylene glycol) was found to be most effective and promising, resulting in long blood circulation times in vivo and other desirable characteristics for the PEGylated SWNTs (PEG-SWNT). There has been much discussion on the potential health effects of carbon nanotubes. Biodistributions of both pristine and functionalized SWNTs in vivo, including their changes over time, have also been determined and reported. However, results are still scarce on the biological consequence of and effects on the functionalized carbon nanotubes, specifically their biodefunctionalization or even any decomposition in vivo. One difficulty in this regard has been a lack of suitable sensitive analytical techniques

In this work, we intravenously administrated PEG-SWNT into mice to study their biodefunctionalization in vivo by using complementary Raman and photoluminescence measurements. There was apparently de-functionalization of PEG-SWNT in liver over time, but not in spleen under similar conditions. The evidence from spectroscopic characterization and analyses is presented, and mechanistic implications are discussed.

Experimental Section

Materials. The SWNT sample from the arc-discharge production method was supplied by Carbon Solutions, Inc. The as-received sample was purified by a combination of thermal oxidation and oxidative acid treatments, as reported previously. Briefly, the sample (1 g) was heated in a furnace to 500 °C in air for 30 min and then refluxed in diluted nitric acid (2.6 M, 500 mL) for 24 h. The solid was collected via centrifugation, washed repeatedly with deionized water until neutral pH, and then dried in a vacuum oven to obtain the purified sample (330 mg). The (3-aminopropyl)triethoxysilane (3-APTES) of molecular weight ∼1500 (PEG3000) was purchased from Alzehir.

The PEG-SWNT sample was prepared as previously reported. Briefly, the purified SWNTs (20 mg) were refluxed in thionyl chloride (5 mL) for 12 h, followed by a complete removal of unreacted thionyl chloride on a rotary evaporator with a vacuum pump. To the treated nanotube sample was added PEG_3000, and the mixture was heated to 120 °C and stirred under nitrogen protection for 3 days. The reaction mixture was cooled to room temperature and then extracted repeatedly with water to obtain PEG-SWNT. The nanotube content in the sample was around 15% by weight according to thermogravimetric analysis (TGA), with a typical nanotube length on the order of 300 nm to 1 μm or so. For animal exposure experiments, the PEG-SWNT sample was dissolved in 0.9% NaCl aqueous solution.

Measurements. TGA was performed on a TA Instruments Q-500 TGA machine. Raman spectra were measured on a Renishaw Raman spectrometer equipped with a 100 W diode laser source for 785 nm excitation and a Jobin-Yvon T64000 spectrometer equipped with a Melles-Griot 35 W He-Ne laser and an Olympus BX-41 microscope. Transmission electron microscopy (TEM) images were obtained on a Hitachi HF-2000 TEM and a Hitachi HD-3000 S-TEM/TEM systems. For Raman measurements, the PEG-SWNT sample was chemically defunctionalized by being boiled in H_2O_2/H_2O (1:1 in volume) mixture until the formation of black soot in the boiling solution. The solid residue was collected by centrifugation at 2800 rpm for 10 min and then dispersed in water via sonication. Both the original PEG-SWNT solution and the defunctionalized sample in aqueous suspension were dropped separately onto glass slides, followed by drying with the use of an infrared lamp. After drying, Raman spectra were recorded as described above.

For Raman specimens from the defunctionalization under conditions simulating those in liver, the PEG-SWNT solution (100 μg carbon equivalent in 10 μL water) was incubated in 1 mL of 5% H_2O_2 solution (0.14 M citrate buffer, pH 7.0) and separately in 1 mL of citrate buffer (0.1 M citrate buffer, pH 4.4), both at 37 °C for 1 week. The resulting suspensions were dropped onto glass slides, followed by drying with the use of an infrared lamp and subsequent acquisitions of Raman spectra.

The livers of two unexposed mice were collected and then homogenized in water to be used as sample background in Raman measurements. The liver homogenate (∼3 g) was divided into two: one mixed with PEG-SWNT (300 μg carbon-equivalent) and homogenized again; and the other mixed with the H_2O_2/H_2O_2 defunctionalized

H_2O_2/H_2O_2 defunctionalized
SWNTs (300 μg carbon-equivalent, in aqueous suspension with 1% Tween 80) and also homogenized agals. Both mixtures were measured on the Resilin-810 Raman spectrometer.

Experiments in Mice. All animal experiments were performed in compliance with the institutional Animal Care and Use Regulations and Guidelines on animal welfare. Male CD-I-C mice (~25 g) were obtained from Peking University Animal Centre, Beijing, China. They were housed in plastic cages (three mice per cage) and kept on a 12 h light/dark cycle. Food and water were provided ad libitum. Following acclimation, mice were randomly divided into groups (three mice per group) for the experiments.

The mice were intravenously injected with 0.3 mL of the PEG-SWNT solution (300 μg carbon-equivalents) per mouse, and those injected with 0.3 mL of 0.9% NaCl solution were taken as the control. The mice were sacrificed at 1 day, 1 week, 4 weeks, and 8 weeks postexposure. The livers and spleens of the exposed and control groups were collected and then homogenized in homogenizers as described above. The homogenates were placed on glass slides and dried under infrared lamp with the full excitation laser power (100 mW) for the liver samples and reduced power (10 mW) for the spleen samples because of the intrinsically stronger fluorescence of the latter.

Results and Discussion

The PEGylated SWNTs were readily soluble in water to form dark-colored homogeneous solutions, and the resulting aqueous solutions (with or without 0.9% NaCl) were stable over an extended period of time (at least several months). The specimen for TEM analysis was prepared by placing a few drops of a dilute aqueous PEG-SWNT solution onto a holey carbon-coated copper grid, followed by drying via evaporation. According to the TEM images (Figure 1), the nanotubes in the PEG-SWNT sample were dispersed either individually or as thin bundles. In high-resolution TEM imaging for SWNTs lying across holes on the holey carbon grid, amorphous materials covering the nanotube surface could be observed (Figure 1), which might be assigned to the PEG-grown moieties.

It is well-known that pristine SWNTs, even after purification with the oxidative acid treatment, exhibit characteristic Raman features including the relatively intense G-band at around 1590 cm⁻¹ (Figure 2A). However, upon covalent functionalization targeting nanotube surface defect-derived carboxylic moieties, such as the PEGylation in this study, the Raman measurements could be subject to overwhelming interference of photoluminescence from the functionalized carbon nanotubes. The luminescence emission is generally brighter in better-functionalized and dispersed nanotubes due to the passivation of nanotube surface defects by the functionalization. In fact, resonance Raman and photoluminescence have been demonstrated as complementary characterization techniques in the evaluation of how well SWNTs are functionalized for their exfoliation into individual or thin bundles of nanotubes. Such a complementary relationship was also confirmed in this study. As shown in Figure 2A, the Raman spectrum of PEG-SWNT is overwhelmed by the luminescence contributions, with the G-band signal barely visible, letting alone the much weaker radial breathing mode (RBM) peaks. Upon the chemical defunctionalization in terms of the HNO₃/H₂O₂ digestion to remove the PEG species from the nanotubes, the Raman features were recovered (Figure 2A). As reported previously, the similar elimination of luminescence interference to recover the intrinsic Raman spectrum of SWNTs could be achieved by thermally defunctionalizing the functionalized nanotube sample (via TGA, for example).

Experiments with simulated biological background were performed to further investigate and demonstrate the complementary relationship between Raman and photoluminescence in PEG-SWNT and the chemically defunctionalized sample. These samples were mixed with the liver homogenate for Raman measurements. As shown in Figure 2B, the mixture with PEG-SWNT exhibited no visible Raman features, but on the other hand, the G-band at around 1590 cm⁻¹ was obvious in the spectrum of the mixture with the chemically defunctionalized sample. It should be noted that with the use of 785 nm laser excitation the autofluorescence from the tissue sample was manageable, hardly prohibiting the sensitive detection of Raman signals. There were also reports in the literature on the Raman
tracking of SWNTs in vivo for both qualitative and quantitative purposes. The simulation experiments here and the results in the literature all suggested that Raman spectroscopy was suitable and adequate for the probing of biodifunctionalization of PEG-SWNT in vivo.

The in vivo experiment involved intravenous administration of the PEG-SWNT solution into mice. The mice were sacrificed at various time points up to 8 weeks post exposure, and the liver and spleen were collected and processed for Raman measurements. For liver samples, no obvious Raman G-band signals were detected in those harvested 1 and 7 days postexposure (Figure 3). However, when PEGylated 14C-enriched SWNTs were used in the same in vivo experiments, nanotubes could be detected 7 or more days postintravenous administration by using the isotopic ratio mass spectrometry technique. Thus, the presence of SWNTs in liver but not detected by Raman suggested that the nanotubes remained well-functionalized.

For the liver sample harvested 4 weeks postexposure, the Raman spectrum clearly exhibited the G-band peak at ~1590 cm⁻¹ and also the RBM band at ~165 cm⁻¹ (Figure 3), with the latter corresponding to the correct average diameter of as-produced SWNTs. The re-emergence of the characteristic Raman features was likely due to biodifunctionalization of the PEGylated SWNTs in liver during the relatively longer period of time postintravenous administration, namely, there were less functionalized or even "free" SWNTs in the liver sample harvested 4 weeks postexposure. Because the PEG chain is generally stable against biotransformation,25 the biodifunctionalization must be at the amide linkages between the PEG functional groups and the nanotubes. Similar Raman features were observed in the liver sample harvested 8 weeks postexposure. This is not a surprise because the presence of unfuctionalized SWNTs in liver at even longer time postexposure is known in the literature.25 Nevertheless, the biodifunctionalization of PEG-SWNT in vivo does not necessarily mean a complete removal of all functional groups, which is not required for the observation of the characteristic Raman peaks from SWNTs. Therefore, the hepatic biodifunctionalization could result in less functionalized or partially PEGylated SWNTs, whose biological fate remains an issue for further investigation.

Interestingly, the biodifunctionalization was absent for the PEGylated SWNTs in spleen. There were no characteristic nanotube peaks in the Raman spectra of all spleen samples harvested up to 8 weeks postexposure (Figure 4). According to the same in vivo experiment with PEGylated 14C-enriched SWNTs (coupled with the quantification by using the isotopic ratio mass spectrometry technique), there were SWNTs in the spleen at least 7 days postexposure. These nanotubes likely remained well-functionalized to hinder the Raman detection. For the spleen sample harvested 8 weeks postexposure, the photoirradiation at 785 nm could result in the re-emergence of the Raman G-band peak (Figure 4). Due probably to some photogenerated thermal defunctionalization effect. Thus, there were indeed PEGylated SWNTs in the spleen over 8 weeks postexposure that were not detected by Raman for the absence of any efficient biodifunctionalization.

The hepatic biodifunctionalization of PEG-SWNT in vivo is probably enzymatic in origin, with combined effects of radical attack and acid hydrolysis.26 In simulation experiments, the PEG-SWNT was incubated separately in 5% H₂O₂ (pH 7.0) or citrate buffer (pH 4.4, the same as in lysosome) for 3 days, simulating the environment for radicals or acid hydrolysis, respectively. The results suggested that there was meaningful defunctionalization in each simulated environment, with the observation of characteristic nanotube peaks in the Raman spectra. Mechanistically, it is possible for PEG-SWNT to enter phagocytic cells via endocytosis, such as phagoctosis or phagocytosis,12,28 first trapped in lysosomes in the cells. Because the function of lysosomes is to allow enzymes to degrade toxic particulate materials,29 there might be radical initiated cleavage or acid hydrolysis reactions in such a cellular environment to be responsible for the observed biodifunctionalization in liver.

The defunctionalization of the covalently PEGylated SWNTs in liver was apparently very slow, in reference to the previously reported in vivo defunctionalization of noncovalently functionalized nanotubes. For SWNTs functionalized via surfactant wrapping, as, for example, the surfactant such as Pluronic F108 absorbed on the nanotube surface could be replaced by serum proteins in rats within 30 min postintravenous administration.30 In the study of Daphnia magna, the lipid coating on SWNTs could be detached and ingested as food source, with the naked nanotubes ejected in 48 h.31 It seems that the mode of nanotube functionalization does make a significant difference in the biological fate of the resulting functionalized nanotube samples.

In summary, the complementary relationship between photolabilenescence and Raman in functionalized SWNTs' and their defunctionalization serves as an effective tool in the study of biodifunctionalization of functionalized SWNTs in vivo. The covalently PEGylated SWNTs could be slowly defunctionalized in liver, but not in spleen, and the hepatic biodifunctionalization
might be enzymatic/radical in origin. The consequences (to the longer term toxicity of functionalyzed carbon nanotubes in vivo, for example) and potential utilities of such biodifunctionalization are interesting topics for further investigations.

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Supporting Information Available. Additional relevant Raman spectra of the liver samples. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes


Single-Walled Carbon Nanotube as a Unique Scaffold for the Multivalent Display of Sugars

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Single-walled carbon nanotube (SWNT) is a pseudo-one-dimensional nanostructure capable of carrying/displaying a large number of bioactive molecules and species in aqueous solution. In this work, a series of dendritic β-D-galactopyranosides and α-o-mannopyranosides with a terminal amino group were synthesized and used for the functionalization of SWNTs, which targeted the defect-derived carboxylic acid moieties on the nanotube surface. The higher-order sugar dendrons were more effective in the solubilization of SWNTs, with the corresponding functionalized nanotube samples of improved aqueous solubility characteristics. Through the functionalization, the nanotube apparently serves as a unique scaffold for displaying multiple copies of the sugar molecules in pairs or quartets. Results on the synthesis and characterization of these sugar-functionalized SWNTs and their biological evaluations in binding assays with pathogenic Escherichia coli and with Bacillus subtilis (a nonvirulent simulant for Bacillus anthracis or anthrax) spores are presented and discussed.

Introduction

Multivalent carbohydrate ligands are known to be considerably more potent than their monovalent counterparts in biological binding−receptor interactions.1,2 Therefore, there has been growing recent interest in various multivalent ligands and their associated configurations for a wide range of applications,3 including the development of high-affinity inhibitors and drugs.4 Among more extensively studied scaffolds for the display of multivalent carbohydrate ligands are dendrimers,5 linear and polydisperse polymers,6 metal nanoparticles,7 polymeric nanoparticles,8 and so on. For example, Stoddart and co-workers synthesized a highly branched carbohydrate dendrimer containing 3−36 peripheral α-o-mannose copies and evaluated their binding affinity to Con A lectin.9 Disney et al. reported the use of a carbohydrate-conjugated fluorescent polymer, poly(propylene ethylenes), to detect pathogenic Escherichia coli.10 For the binding with E. coli, Wu and co-workers attached α-mannose moieties to gold nanoparticles to target selectively the type 1 pill of the ORN178 strain.11 Gu et al. developed sugar-coated polystyrene nanobeads for the binding and agglutination of E. coli cells.12

Single-walled carbon nanotubes (SWNTs) have attracted much recent attention for potential biological applications,13−18 including their functionalization with various bioactive species such as carbohydrates,19 DNA,20 proteins,21,22 and peptides.23 The unique pseudo-one-dimensional structure of a SWNT has been exploited to serve as a linear and semiflexible nanoscale carrier for displaying multiple copies of biomolecules as specific interactions with cells. A representative example is due to Eklund et al.,14 who used the bovine serum albumin-functionalized SWNTs in conjunction with E. coli-specific antibody to capture the pathogen in physiological solution. In another example, Gu et al. solubilized SWNTs via covalent functionalization with the derivatized 2-aminoethyl-β-D-galactopyranoside (Scheme 1) in likely the amidation of nanotube-bound carboxylic acids.15 These β-D-galactose-functionalized nanotubes (Gln-SWNT), each displaying multiple copies of the sugar, were found to have adhesion to pathogenic E. coli O157:H7 to result in significant cell agglutination.17 Recently, Wang et al. found that monosaccharides (β-D-galactoses or α-o-mannoses)-functionalized SWNTs bind to and aggregate effectively Bacillus anthracis (Sterne) spores in the presence of a divalent cation (such as Ca2+) and that the binding is unique to the nanotube-displayed carbohydrates.18

In the functionalization of nanotubes with tethered β-D-galactoses and α-o-mannoses, the carboxylic acid moieties on the nanotubes, which are resulted from the oxidation of the surface defects, have been targeted.19 With limited defect sites on the nanotube surface, a higher population of multivalent ligands could be attained by using sugar dendrons (Scheme 1) in the nanotube functionalization. The resulting larger number of displayed ligands per nanotube also corresponds to significantly improved aqueous solubility of the functionalized nanotube samples,20 which not only enhances biocompatibility but also enables more quantitative characterization for a better understanding of the structural details on the nanotubes displaying multivalent carbohydrate ligands. Here we report the synthesis and characterization of dendritic β-D-galactopyranosides and α-o-mannopyranosides for the functionalization of SWNTs (Scheme 1). As the functionality varies from mono- to bis- to tetra-, the population of the sugar moieties in the functionalized nanotube sample increases, as are improved solubility and related properties. Results from the biological evaluation of these sugar-functionalized SWNTs in binding assays with pathogenic E. coli and with Bacillus subtilis (a nonvirulent simulant for Bacillus anthracis or anthrax) spores are presented and discussed.
**Experimental Section**

**Materials.** 1-BuNt bromoacetate, N-hydroxy succinimide, trifluoroacetic acid, triethylphosphine, tri-(4-fluorophenyl) triazene, and N,N-dicyclohexyl carbodiimide were purchased from Sigma-Aldrich. 3,5-Dihydroxybenzyl alcohol, 18-crown-6, N,N-dicyclohexyl carbodiimide, β-D-galactose-pentaacetate, α-D-mannose, and anethole were obtained from Acros. N-Bromosuccinimide was supplied by ACOGADO Research Chemicals, Ltd. Solvent grade THF was dried and distilled over molecular sieves and then distilled over sodium before use. Other solvents were either spectrograde/melting point grade or purified via simple distillation. Deuterated NMR solvents were obtained from Cambridge Isotope Laboratories.

The SWNT sample from the arc-discharge production was supplied by Carbon Solutions, Inc. It was purified by using a combination of thermal oxidation and oxidative acid treatment. In a typical experiment, a nanotube sample (1 g) was thermally oxidized in air in a furnace at 300 °C for 30 min. After the thermal treatment, the remaining sample was added to an aqueous HNO₃ solution (2.6 M), and the mixture was refluxed for 48 h. Upon centrifuging at 1500 g (Fisher Scientific Centric 225 Centrifuge), the supernatant was discarded and the remaining solids were washed with deionized water until neutral pH and then dried under vacuum. Acid-base titration was used to estimate the population of carboxylic acid groups in the purified sample. Briefly, a portion of the sample was stirred in aqueous Na₂CO₃ solution (0.05 M, 50 mL) for 3 days. Then, the nanotubes were removed via vigorous centrifuging, and the resulting solution was diluted 1:1 to 5:1. One aliquot (50 mL) was mixed with aqueous HCl solution (0.05 M, 10 mL), and the mixture was boiled for 30 min to remove CO₂ completely, followed by the addition of excess HCl with aqueous NaOH (0.05 M).

**Measurements.** NMR measurements were carried out on a JEOL Eclipse +500 NMR spectrometer and a Bruker Advance 500 NMR spectrometer equipped with a high-resolution magic-angle-spinning (HR-MAS) probe designed specifically for gel-phase NMR. Matrix-assisted laser desorption ionization-time-of-flight (MALDI-ToF) MS was performed on a Bruker AutoFlex system, and 2,5-dihydroxybenzoic acid was used as the sample matrix. Thermogravimetric analysis (TGA) results were obtained on a TA Instruments Q500 TGA, with a scanning rate of 10 °C/min for measurements in nitrogen or air. Optical absorption spectra were recorded on a Shimadzu UV-3600 UV/Vis/NIR.
Table 1. Results on Man−, Man−, and Man− in Functionalization/Solubilization of SWNTs

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<thead>
<tr>
<th>molar ratio of sugar−NH$_2$ to nanotube−COOH</th>
<th>starting SWNTs solubilized (%)</th>
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<tr>
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<tr>
<td>2:1</td>
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*Some might argue that nanotubes are dispersed, not solubilized. However, in the literature, the kind of functionalization used in this work is generally referred to as solubilization.

12 h. The precipitate was collected via filtration and then dried in a vacuum oven to yield the hydrolyzed product 1 as a white solid (1.1...
Figure 1. Comparisons for (a) the $^1$H NMR spectra of Gal$_2$-SWNT (top, solution phase; middle, gel phase) and Gal$_2$N$_2$ (bottom); and (b) the $^{13}$C NMR spectra of Gal$_2$-SWNT (top, with inset) and Gal$_2$N$_2$ (bottom), all in D$_2$O. The peaks marked with * were due to residual methanol in the sample.

Figure 2. Comparisons for (a) the $^1$H NMR spectra of Gal$_2$-SWNT (top) and Gal$_2$N$_2$ (bottom); and (b) the $^{13}$C NMR spectra of Gal$_2$-SWNT (top, with inset) and Gal$_2$N$_2$ (bottom), all in D$_2$O. The peaks marked with * were due to residual methanol in the sample.
Figure 3. Optical absorption spectra of (a) Gal₆-SWNT, (b) Gal₁₆-SWNT, (c) Man₁₆-SWNT, and (d) Man₆-SWNT in D₂O solution (solid line) and in the solid-state on glass substrates (dashed line). For the spectral comparison between solution-phase and solid-state, the spectra in each corresponding pair were normalized by multiplying factors to the spectra to make their absorbance values the same at a selected wavelength (1000 nm for Man₁₆-SWNT as an example).

Figure 4. Raman spectra (633 nm excitation) of the functionalized SWNTs and the starting purified nanotube sample are compared.

Compound 3. There were two steps (Scheme 3), with the first step being the conversion of the bromide to 1 to iodide (1°). Compound 1 (3.01 g, 7 mmol) and sodium iodide (10 g, 67 mmol) were refluxed in acetone (50 mL) for 12 h. The reaction mixture was allowed to cool, and then the solvent was evaporated completely. The residue was partitioned between water and chloroform. The organic layer was collected and dried with activated MgSO₄, followed by the removal of solvent to yield 1° (3.56 g, quantitative). H NMR (500 MHz, CDCl₃) δ 6.53 (d, 2H), 6.37 (t, 1H), 4.48 (s, 4H), 4.35 (s, 2H), 1.50 (s, 18H).

Figure 5. SEM images of a Gal₁₆-SWNT specimen (ultrathin film): (a) at the edge of the specimen and (b) in the fractured portion of the specimen.
Scaffold for the Multivertex Display of Sugars

**Figure 6.** SEM image of the GaN-SWNT spacers (on carbon-coated copper grid) prepared from a very dilute solution sample.

ppm: 13C NMR (125.7 MHz, CDC13) δ 167.4, 159.17, 141.42, 106.16, 101.73, 62.64, 65.88, 25.16, 5.14 ppm.

(a) Incongruent dissolution
155(kg) Na2CO3 (0.3 mmol) and 125(kg) K2CO3 (2.4 mmol) and 180-krown6 (172 mg, 0.7 mmol) in arctic (30 mL) was prepared, and to the solution was added 1.5 (3.5 g, 7.7 mmol).

After filtering for 36 h, and then the reaction solvent, the reaction mixture was separated on silica gel column (first heptane—ethyl acetate at 1:9 and then methanol—ethyl acetate at 1:9) to afford 8(Gal) as white solids (723 mg, 67% yield).

**1H NMR (300 MHz, CDCl3) δ 6.94 (d, 2H, 6.96 (t, 2H, 6.95 t, 1H), 5.32–5.29 (ct, 4H), 5.24–5.18 (m, 8H), 4.97 (t, 4H), 4.97 (s, 4H), 4.31 (s, 4H), 3.83 (s, 4H), 3.26–3.24 (d, 2H, 6.94–6.19 (m, 4H), 2.95–2.93 (d, 4H), 1.92 (s, 12H) ppm. 13C NMR (125.7 MHz, CDCl3) δ 170.44, 170.26, 170.18, 169.55, 167.89, 158.79, 158.71, 140.13, 149.02, 108.31, 107.05, 102.20, 101.16, 101.29, 70.83, 70.77, 69.59, 68.78, 68.55, 67.47, 67.03, 61.31, 38.85, 33.40, 20.75, 20.72, 20.70, 20.65 ppm. MALDI-TOF MS (M + Na+) 2195.56 (theoretically, 2193.60).

The same procedure was applied to the synthesis of 8(MOM) (1.37 g, 63% yield).

**1H NMR (300 MHz, CDCl3) δ 7.06 (s, 4H, NH), 6.08 (d, 4H), 6.60 (d, 2H), 6.56 (s, 2H), 6.57 t (1H), 5.32–5.29 (ct, 4H), 5.24–5.18 (m, 8H), 4.97 (t, 4H), 4.97 (s, 4H), 4.31 (s, 4H), 3.83 (s, 4H), 3.26–3.24 (d, 2H, 6.94–6.19 (m, 4H), 2.95–2.93 (d, 4H), 1.92 (s, 12H) ppm. 13C NMR (125.7 MHz, CDCl3) δ 170.44, 170.26, 170.18, 169.55, 167.89, 158.79, 158.71, 140.13, 149.02, 108.31, 107.05, 102.20, 101.16, 101.29, 70.83, 70.77, 69.59, 68.78, 68.55, 67.47, 67.03, 61.31, 38.85, 33.40, 20.75, 20.72, 20.70, 20.65 ppm. MALDI-TOF MS (M + Na+) 2195.56 (theoretically, 2193.60).

**Gal-NH2 and MeGal-NH2.** The first step was to convert the bromide in 5 to azide (Gal-‘N3 and MeGal-‘N3). For Gal-‘NH2, a solution of 5(Gal) (724 mg, 0.34 mmol) in acetone (20 mL) was prepared and to the solution was added sodium azide (622.6 g, 3.44 mmol) and 18-crown-6 (54 mg, 6.3 mmol). The mixture was refluxed for 12 h and then cooled to room temperature. The solvent was evaporated, and the residue was partitioned between water and ethyl acetate. The aqueous layer was extracted with ethyl acetate twice. All of the ethyl acetate fractions were combined and dried with anhydrous MgSO4. The solvent was evaporated, and the residue was dried in a vacuum oven to yield Gal-‘NH2 as a white solid (715 mg, quantitative).

**1H NMR (300 MHz, CDCl3) δ 6.78 (8, 4H, NH), 6.65 (4H, 6.71 (0, 0H), 6.69 (4H, 6.59 (3, 0H), 6.52 (1H, 6.51 (2H, 6.50 (2H, 4.66 (s, 8H), 4.49 (8H, ppm. 13C NMR (125.7 MHz, CDCl3) δ 170.44, 170.26, 170.18, 169.55, 167.89, 158.79, 158.71, 140.13, 137.98, 107.32, 106.97, 102.17, 101.89, 101.25, 70.81, 70.87, 69.77, 69.79, 68.53, 67.63, 67.61, 61.32, 61.32, 28.66, 20.72, 20.70, 20.65 ppm. MALDI-TOF MS (M + Na+) 2157.47 (theoretically, 2156.69).

The same procedure was applied to the synthesis of MeGal-‘NH2 (1.02 g, quantitative).

**1H NMR (300 MHz, CDCl3) δ 7.01 (4H, 6.64 (d, 4.4H, 6.53 (4H, 6.51 (3H, 6.49 (2H, 6.39 (2H, 6.37 (1H, 6.31 (1H, 6.19 (2H, 6.18 (H, 5.22–5.17 (m, 8H), 4.95 (4H, 4.78 (d, 4H, 4.64 (s, 4H, 4.30(d, 4H, 3.93–3.89 (m, 4H, 3.79–3.74 (m, 4H, 3.63–3.57 (m, 4H, 3.57–3.54 (m, 4H, 2.08 (s, 12H, 2.07 (s, 12H, 1.93 (s, 12H, 1.91 (s, 12H) ppm. 13C NMR (125.7 MHz, CDCl3) δ 170.44, 170.26, 169.15, 169.76, 160.00, 160.05 158.51, 139.99, 137.55, 107.35, 107.03, 101.95, 101.99, 97.66, 66.59, 69.43, 69.03, 68.91, 67.30, 66.50, 66.10, 65.24, 54.77, 38.45, 20.75, 20.70, 20.65 ppm. MALDI-TOF MS (M+Na+) 2134.08 (theoretically, 2133.69).

For Gal-‘NH2, a solution of 5(Gal) (715 mg, 0.34 mmol) in THF/ONa/CH3OH (0.05 M, 3 mL) was prepared. After the solution was stirred for 12 h, Amberlite IR-120(G) ion-exchange resin was added to adjust the pH to 7.0. The resin was removed via filtration, and the solution was dried with anhydrous MgSO4, followed by solvent evaporation to yield Gal-‘NH2 as a white solid (400 mg, quantitative).

**1H NMR (300 MHz, CDCl3) δ 6.47 (s, 4H, 6.43 (2H, 6.36 (2H, 6.26 (1H, 6.23 (1H, 4.63 (4H, 4.32 (d, 4H, 4.36 (s, 4H, 4.13 (t, 3H, 3.97–3.94 (m, 4H, 3.77–3.74 (m, 4H, 2.08 (s, 12H, 2.07 (s, 12H, 1.93 (s, 12H, 1.91 (s, 12H) ppm. 13C NMR (125.7 MHz, CDCl3) δ 170.33, 159.21, 158.31, 139.53, 138.37, 137.24, 106.85.
Table 2. Compositions in the Functionalized Nanotube Samples and Related Parameters

<table>
<thead>
<tr>
<th></th>
<th>sugar content (%)</th>
<th>SWNT content (%)</th>
<th>avg No. of nanotube carbon per sugar unit</th>
<th>aqueous solubility (SWNT-equivalent, mg/mL)</th>
<th>starting SWNTs solubilized (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gal-SWNT</td>
<td>45</td>
<td>43</td>
<td>14</td>
<td>0.83</td>
<td>11</td>
</tr>
<tr>
<td>Man-SWNT</td>
<td>47</td>
<td>42</td>
<td>13</td>
<td>0.84</td>
<td>22</td>
</tr>
<tr>
<td>Galg-SWNT</td>
<td>46</td>
<td>19</td>
<td>6</td>
<td>1.7</td>
<td>20</td>
</tr>
<tr>
<td>Man@SWNT</td>
<td>45</td>
<td>18</td>
<td>6</td>
<td>1.0</td>
<td>25</td>
</tr>
<tr>
<td>Gal@SWNT</td>
<td>44</td>
<td>27</td>
<td>9</td>
<td>3.1</td>
<td>27</td>
</tr>
<tr>
<td>Man@SWNT</td>
<td>45</td>
<td>29</td>
<td>11</td>
<td>4.3</td>
<td>30</td>
</tr>
</tbody>
</table>

*The remaining (100% - sugar content – SWNT content) is the content of linkers/antennae. *a The degree of functionalization exceeding that for the estimated COOH population might be due to preferable solubilization of those nanotubes with more than average COOH contents and also some noncovalent but strong adsorption of the amine.

103.18, 101.40 (2C), 75.15, 72.79, 70.76, 69.19, 68.59, 68.33, 66.41, 60.95, 54.02, 39.07 ppm. MALDI-TOF MS (M + Na)+ 1484.30 (theoretically 1484.52). The same procedure was applied to the quantitative conversion of Meα2N2 to MeαN2, 2H NMR (500 MHz, D2O) δ 6.4 (d, 4H), 6.32 (d, 2H), 6.34 (s, 2H), 4.75 (s, 4H), 4.61 (s, 4F), 4.24 (s, 4H), 4.05 (s, 4H), 3.81 (m, 4H), 3.76 (s, 4E), 3.70–3.75 (m, 12H), 3.20–3.25 (m, 4H), 2.70 (m, 8H), 1.85–1.92 (m, 38H) ppm. 13C NMR (125.7 MHz, D2O) δ 166.42, 159.23, 158.34, 136.13, 138.36, 107.43, 106.39, 101.87, 101.30, 99.67, 72.88, 70.64, 70.10, 69.20, 66.69, 65.60, 65.70, 65.09, 65.54, 64.92, 34.81 ppm. MALDI-TOF MS (M+Na)+ 1461.38 (theoretically 1461.53).

Nanotube Functionalization. Before the functionalization reaction, Galα2N2 and Manα2N2 were reduced to Galα-NH2 and Manα-NH2 by the classical palladium-catalyzed hydrogenation. At 0 °C, to a solution of Galα-NH2 or Manα-NH2 (80 mg, 0.35 mmol) in methanol–H2O (10 mL) was added Pd/C (10 wt % palladium on activated carbon, 70 mg). The reaction mixture was gradually warmed to room temperature and stirred with the purging of hydrogen gas for 4 h. The Pd/C was removed by filtration, and the filtrate was evaporated to obtain Galα-NH2 or Manα-NH2.

In the functionalization of SWNTs with Galα-NH2, a purified nanotube sample (20 mg) was mixed with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDAC, 112 mg, 60 mmol) in aqueous KH2PO4 buffer (45 mL, pH = 7.4). After sonication for 2 h, the freely prepared Galα-NH2 (55 mmol) was added. After sonication for another 36 h, the reaction mixture was loaded into a membrane tubing (cutoff molecular weight – 12000) for dialysis against deionized water for 3 days. The suspension from the dialysis was centrifuged at 3000 g for 30 min to obtain a dark but optically transparent solution of Galα-

SWNT. 1H NMR (500 MHz, D2O) δ 7.27 (broad), 6.61 (s, broad), 6.55 (broad), 6.50–6.40 (broad), 4.52 (s, broad), 4.35 (d, broad), 3.58 (s, broad), 3.93 (d, broad), 3.76 (s, broad), 3.64 (broad), 3.54 (broad), 3.50 (broad) ppm. 13C NMR (125.7 MHz, D2O) δ 170.99, 158.48, 106.61, 101.17, 75.14, 72.84, 77.97, 65.58, 68.60, 63.36, 66.60, 60.94, 39.06 ppm. Similarly, for Manα-SWNT. 1H NMR (500 MHz, D2O) δ 7.35–7.42 (broad), 4.35–4.10 (broad), 3.91 (s, broad), 3.85 (d, broad), 3.77 (d, broad), 3.67 (t, broad), 3.55–3.40 (broad), 3.43 (broad) ppm. 13C NMR (125.7 MHz, D2O) δ 170.11, 159.49, 158.46, 105.95, 106.98, 101.21, 99.66, 72.87, 70.61, 70.10, 69.18, 66.70, 66.45, 65.66, 65.93, 38.58 ppm.

Sugar Test. The spectrophotometric method with the anthrone reagent was used to determine sugar contents. In a typical experiment, a solution of sugar-functionalized SWNTs (50 μL) was mixed with deionized water (50 μL), HCl (3%, 1 mL), formic acid (0.3 mL), and anthrone reagent (8 mL, 0.2 mg/mL in 80% H2SO4). The solution mixture was kept in a boiling water bath for 12 min and then rapidly cooled in an ice bath for 30 min. Afterwards, the UV/vis absorption of the mixture was measured and corrected for the solution absorption preheating (before the coloration process). Separately, the standard sugar (β-D-galactose or α-D-mannose) solutions of known concentration (1.0 mg/mL) were prepared. Various aliquots of the standard solutions (5, 25, 50, 75, and 100 μL) were tested by using the same procedure above to establish standard curves, from which the sugar content in the functionalized nanotube sample was obtained.

Assay for E. coli. The green fluorescent protein (GFP)-expressing E. coli/O157:H7/WT/W0017 was transformed through electroporation.26 The freeze E. coli samples were removed and cultured on tryptic soy agar (TSA) plates supplemented with lansacyn (50 μg/mL). After overnight growth at 37 °C, the bacteria were harvested and washed with phosphate buffer solution (PBS) via the centrifuging-suspending cycle three times. Finally, the E. coli cells were suspended in PBS to an optical density of 0.6 at 600 nm (10–15 cells/mL).

In cell adhesion and precipitation experiment, a suspension (25 μL) of E. coli O157:H7/WT/W0017 cells was 2-fold serially diluted and incubated with a solution (10 μL) of either Gal-SWNT (0.14 mg/mL) or Gal@SWNT (0.13 mg/mL) for 3 h at room temperature with gentle inversions of the tubes at 15 min intervals. The precipitates at the tube bottom were collected and wet-mounted onto a glass slide for microscopy analyses.

For the colony-forming unit (CFU) reduction assay, the same procedure described above was used and at the end of incubation the mixture was centrifuged with a few force (300 g) for 30 s. The supernatant was collected and 10-fold serially diluted. At each dilution, 100 μL was plated (in triplicate) onto TSA agar plates by using spread plate technique. The plates were incubated overnight at 37 °C. CFU was counted, and the percentage of CFU reduction was calculated and compared to that of the control without the sugar-functionalized SWNTs.

Assay for B. subtilis Spores. B. subtilis spores (strains ATCC33234) were supplied by American type Culture Collection (Manassas, VA) and the spore suspension was prepared by following established procedures. In a typical binding experiment, an aqueous suspension of B. subtilis spores (40 μL, 2.6 × 108 CFU/mL) was mixed with a solution of α-D-mannose-functionalized SWNTs (40 μL, 0.2 mg/mL α-D-mannose equivalent concentration) or with distilled water (40 μL) as control, followed by the addition of aqueous CaCl2 (20 μL, 1.0 M). The mixtures (sample and control) were rotated for 12 h. A small aliquot (10 μL) of the sample (or control) was dropped onto a glass slide (covered with cover glass slide) for optical microscopy analyses.

Results and Discussion

The sugar dextrans Galα-NH2, Manα-NH2, Galα-NH2, and Manα-NH2 were prepared by using boronated benzoxazine (3,5-dihydroxybenzyl alcohol and 1) as building blocks in classical elimination and carbohydrate-activated amiation reactions (Scheme 2), with generally high product yields. The synthetic strategy was thus that the dendritic framework was constructed first before being coupled with the armine-terminated monosaccharide. A significant advantage of the strategy was that sugar molecules were involved only in the end steps, thus difficulties associated with the sugar deprotection and sterelcondensation were avoided.

The same carbohydrate-activated amiation was used in the functionalization of SWNTs with the dextrin, with the tethered
amino groups coupling with the surface defects-derived carboxylic acids on the nanorubes. The functionalization reaction was heterogeneous in nature, so that at typical an excess amount of the functionalization agent was used. For the functionalization with 2'-aminoethyl-d-o-mannopyranoside (Man-), the molar ratio of Man- to the nanotube-bound carboxylic acids (estimated at 5% mole fraction of the nanorube carboxy groups based on titration results) was varied. The results in Table 1 show that the amount of SWNTs solubilized in the functionalization reached a plateau at the Man- to acid molar ratio of 5 or so. Thus, at the molar ratio of 100, the amount of solubilized nanotubes should represent the limit for the functionalization agent under the otherwise defined experimental parameters and conditions. As shown in Table 1, the percentage of the starting purified SWNTs solubilized in the functionalization reaction increased from about 25% for Man- to 35% for Man- and to 50% for Man-.

The higher-order sugar dendrons were obviously more effective in the functionalization and solubilization of SWNTs. The sugar dendron-functionalized carbon nanotube samples, Gal2-SWNT, Man2-SWNT, Gal2-SWNT, and Man2-SWNT, are all readily soluble in water (see note in Table 1), allowing their being investigated with solution-phase techniques. The first was the characterization of the samples by NMR, both in solution and in the gel phase. For example, the 1H NMR spectra of...
Ga2-SWNT and Ga2-N2 in solutions are compared in Figure 2a. The signals of the nanotube sample are obviously significantly broader than those of the corresponding free dendron, which may be attributed to the high molecular weight and low mobility of carbon nanotubes. For the gel-phase NMR measurement of the same Ga2-SWNT sample in a high resolution-magic angle spinning (HR-MAS) probe specifically for the gel-phase, the 1H spectrum is better resolved, with the signal patterns in the sugar region comparable with those of the free dendron (Figure 1a). The aromatic proton signals for Ga2-SWNT are slightly shifted downfield, 6.82 ppm and 6.70 ppm for the nanotube-bound dendron from 6.72 ppm and 6.66 ppm for the free dendron, respectively, which might be due to the formation of amide linkages affecting the aromatic ring in the close vicinity. The same effects could be reasonable (for the absence of the benzyl proton signal in the spectrum of Ga2-SWNT because the proton is right next to the nanotube). This proton has a chemical shift of 4.36 ppm in the spectrum of Gal2-N2. It is known in the literature that the effect of large aromatic ring currents in carbon nanotubes on the local magnetic environment of protons in close proximity causes their resonance to either shift into the region where it is difficult to be identified or to become too broad to be detected.

Similar 1H NMR results, including effects on the signal broadening and shifting, were found for other dendron-functionalized SWNTs. Shown in Figure 2a is a similar comparison between the 1H NMR spectra of Gal2-SWNT and Gal2-N2 in solutions, and the comparison between Man4-SWNT and Man4-N2 is largely the same.

13C NMR spectra of the dendron-functionalized SWNTs, especially Ga2-SWNT and Man4-SWNT, were readily obtained in solutions, unlike those of gal2-SWNT and man4-SWNT whose relatively poor solubility (thus low solution concentrations) made their 13C NMR measurements rather difficult. For the comparisons between Ga2-SWNT and Gal2-N2 (Figure 1b) and between Gal2-SWNT and Gal2-N2 (Figure 2b), there are signal broadening effects from free dendron to those attached to SWNTs, though relatively less so from Ga2-N2 to Ga2-SWNT as one would expect. There are C4 carbon resonances in both Gal2-SWNT (Figure 1b) and Gal2-SWNT (Figure 2b) spectra, and the latter also exhibits some of the tether carbon signals due to the higher solution concentration and perhaps also to these carbons being further away from the nanotubes (less affected by their large ring currents).

The optical absorption spectra of the functionalized SWNTs were measured both in solution (only down to 1500 nm because of the overwhelming interference from D2O beyond that) and in the solid state (samples deposited on the surface of glass slides). The S11 (~1850 nm) and S21 (~1560 nm) bands due to van Hove singularity transitions in semiconducting SWNTs and the weak M1 transition (~740 nm) in metallic SWNTs were all present in the spectra of dendron-functionalized nanotube samples (Figure 3), suggesting the electronic properties of the nanotubes were largely preserved in the functionalization targeting defects-derived carboxylic acid moieties on the nanotube surface, as also observed in many other such functionalization schemes.

The solution spectra of Ga2-SWNT and Man4-SWNT were somewhat better resolved than their solid-state counterparts. For Ga2-SWNT and Man4-SWNT, the generally weaker absorption bands in the solution spectrum were probably a simple result of their lower solubility (thus lower solution concentrations).

The optical absorption spectra of the functionalized SWNTs were characterized by Raman with 532 nm excitation. Interestingly, unlike in other well-functionalized nanotube samples, no substantial luminescence interference was observed. Thus, resonant Raman spectra could be measured for all of the dendron-SWNT samples without their being thermally or chemically functionalized first. As compared in Figure 4, the Raman spectra of the different dendron-SWNT samples are largely similar among themselves and also similar to that of the starting purified nanotube sample, exhibiting the typical radial breathing mode peaks around 140 and 160 cm⁻¹, D-band around 1350 cm⁻¹, tangential G-band around 1580 cm⁻¹, and D*-band around 2600 cm⁻¹.

Scanning electron microscopy (SEM) technique was used to examine the morphology of sugar dendron-functionalized nanotube samples. Shown in Figure 5 are SEM images for the Ga2-SWNT sample, for which the specimen was prepared by drop-casting an ultrathin film from the sample solution. Abundant nanotubes were observed, with their being randomly oriented at the edge of the specimen (Figure 5a) but more ordered in the fractured portion of the same specimen (Figure 5b). For Ga2-SWNT, a very dilute aqueous solution was used to prepare the SEM specimen (depositing a few drops and then evaporating the water). The image shows generally dispersed nanotubes (Figure 6).

The thermal defunctionalization behavior of the dendron-SWNT samples was very different from those of many other functionalized carbon nanotubes. The functional groups (sugar dendrons) could not be removed from the nanotubes in thermogravimetric analysis (TGA) scans (under inert atmosphere at 800 °C) and, instead, they were carbonylated under the thermal defunctionalization conditions. For TGA in air, the carbon nanotubes were burnt at temperatures similar to those for the sugars. Therefore, the usually effective TGA estimate of nanotube contents in the functionalized samples was not applicable, and the compositions in dendron-SWNT samples were determined in terms of sugar analyses.

The sugar contents in the dendron-SWNT samples were quantified by spectrophotometry with the anthrone reagent. Results thus obtained for the sample compositions are shown in Table 2. There are no meaningful differences in sample compositions between Gal- and Man4-Gal2- and Man4-, or Gal2- and Man4- as functional moieties, despite the fact that the Gal-based functionalization agents are consistently less effective (a lower percentage of starting purified SWNTs solubilized) than their Man-based counterparts in the nanotube solubilization (Table 2). The power solubilization performance of the Gal-based agents is probably due at least in part to the significantly lower (by a factor of 3 to 4) aqueous solubility of β-D-galactoside than that of α-D-mannose.

The known sample compositions also allow the calculation for the average number of sugar units per carbon in the samples (Table 2). From Gal- (or Man4-) to Gal2- (or Man4-), the number decreases significantly, namely, that more sugars are displayed on the same amount of SWNTs. For Ga2-SWNT and Man4-SWNT, even though there is no advantage in the number of sugars displayed (because of the relatively larger amount of tethering moieties for the display), the improved aqueous solubility and the specific configuration of β-D-galactoside or α-D-mannose displayed in quartz may offer properties that are not available to other configurations.

It has been reported that Ga2-SWNT binds to E. coli O157:H7 to result in significant cell agglutination. The same assay was used to evaluate the binding of Ga2-SWNT with the same E. coli strain. As shown in Figure 7, there was obviously significant aggregation of the cells in the presence of Ga2-SWNT. The amount of aggregates (precipitates at the bottom
of the centrifuge tube was larger for a higher starting E. coli concentration of 10^6 cells/mL than that of 5 x 10^5 cells/mL (Figure 7). Between Gal-SWNT and Gal-SWNT under similar experimental conditions, the amount of recovered aggregates was clearly larger in the former than in the latter (Figure 7). This was reflected more quantitatively in results from the CP assay. As compared in Figure 8, Gal-SWNT was obviously more effective in the aggregation of E. coli CI057: H7 cells to result in a more significant CP reduction. Mechanistically, the binding responsible for the cell aggregation is attributed to specific ligand—receptor interactions of the nanotube-displayed β-D-galactosides with E. coli surface galactos-binding-protein.12,13 The more favorable binding by Gal-SWNT seems to suggest that the paired β-D-galactosides could be more effective in the specific interactions. However, the multivalent binding of carbohydrates with cell surface receptors is a well-established phenomenon.12,13 A more definitive conclusion requires more systematic investigations with these and other assays.

The complexity with desired quantitative evaluations of the sugar-functionalized SWNT's in binding assays is also reflected in the results on B. subtilis spores. B. subtilis is a commonly used nonviral simulant for Bacillus anthracis (anthrax),14 with surface expressed with various carbohydrates.15 As reported previously for B. anthracis (Sterne) spores,16 Man-SWNT also bound to B. subtilis spores for their significant aggregation in the presence of calcium cation (Figure 9). Similarly, Man-, Man-Na-, and Man-SWNT were both capable of binding and aggregating B. subtilis spores in the same assay, as also illustrated in Figure 9. However, a more quantitative comparison of the different α-D-mannose—functionalized SWNT's samples was hindered by other issues beyond simply the different displays of α-D-mannoses in Man-, Man-Na-, and Man-SWNT. For example, it was found that Man-SWNT samples of somewhat different Man/nanotube ratios (from slight variations in reaction conditions in the synthesis) their bindings with the spores (and the associated degrees of aggregation) were obviously different. Such effects were more pronounced in the binding assay of Man-SWNT with B. subtilis spores in the presence of calcium cation. Especially for Man-SWNT samples of slightly higher Man/nanotube ratios, the binding assay resulted in well-dispersed aggregates of an irregular rod-like shape (20–50 μm in length and 5–10 μm in diameter according to optical microscopy analyses). We speculate that some of these complications might be due to the divergent cation-mediated interactions between the paired α-D-mannoses in Man-Na-SWNT and Man-SWNT, which could be competing with the binding with cell surface receptors. Further investigations are required for a better understanding of the results.

Summary and Conclusion

A series of dendritic β-D-galactopyranosides and α-D-mannopyranosides and used for the functionalization of SWNT's targeting the defect-derived carbonyl acid moieties on the nanotube surface. The functionalized nanotube samples were characterized by using established NMR, optical spectroscopy, and microscopy techniques. The results suggest that the higher-order sugar dendrons are more effective in the solubilization of SWNT's, with the corresponding functionalized nanotube samples of improved aqueous solubility characteristics, and that the nanotube is indeed a unique pseudo-one-dimensional scaffold for displaying multiple copies of the sugar molecules in pairs or quarts. These multivalent carbohydrate configurations may potentially offer interesting chemical and biochemical properties and functions, despite some of the complications that are yet to be understood (as reflected in the results of the binding assay with B. subtilis spores).

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Supporting Information Available. Synthesis of Gal-Na, Man-Na, and man-1. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(16) According to the MSDS data sheets (www.fibernet.com).
Covalently PEGylated Carbon Nanotubes with Stealth Character In Vivo**

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Since their discovery, carbon nanotubes have attracted tremendous research interest for their unique physicochemical properties and promise in biomedical applications.\[1\] Notably, there has been much discussion of drug-delivery systems based on carbon nanotubes, with some progress achieved in vitro.\[3\] For potential in vivo applications, however, a significant limitation has been identified with the pharmacokinetic behavior of carbon nanotubes, owing to their rapid clearance or high hepatic uptake.\[4-8\] During the process of our reported work, Liu et al. reported that polyethylene glycol (PEG) could be used to wrap single-walled carbon nanotubes (SWNTs) to escape the capture of reticuloendothelial system (RES) for a blood circulation half-life of up to two hours.\[8\] We found that the covalent PEGylation of SWNTs is much more effective to achieve their prolonged blood circulation and low hepatic uptake. In fact, the observed blood circulation half-life ($t_{1/2}$) of covalently PEGylated SWNTs (PEG-SWNTs) is many fold longer than the current value in the literature.\[8-10\] Making them stealth nanotubes. In this Communication, experimental results from blood circulation and biodistribution studies are reported and the possible mechanism for the stealth character of PEG-SWNTs discussed. Also highlighted are preliminary results on tumor uptake of PEG-SWNTs in vivo, which are relevant to potential applications of the stealth nanotubes in the drug-delivery systems.

The skeleton $^{13}$C-enriched SWNTs (from laser-ablation production, $^{13}$C-content 26.12%) were covalently functionalized with diamine-terminated PEG oligomers (average molecular weight $\approx$1500, PEG$_{1500}$)\[11\] and the resulting PEG-SWNTs were used for in vivo experiments and quantitative measurements with isotope ratio mass spectrometry (isotope-MS).\[12-15\] The PEGylated SWNTs were clearly visible in transmission electron microscopy (TEM) images (Figure 1, where the thin bundles of 10-20 nm in diameter and 1-1.5 μm in length were due to the aggregation of functionalized SWNTs in the preparation of specimens for TEM analysis). The PEG-SWNTs sample was readily soluble in water and the resulting aqueous solution with 0.9% NaCl was stable for at least six months. The nanotube content in the aqueous solution for in vivo experiments was determined by isotope-MS.

In the plasma pharmacokinetic study, mice were injected intravenously with the solution of PEG-SWNTs. These PEGylated SWNTs exhibited an impressively long blood circulation time. The concentrations of PEG-SWNTs in blood at different time intervals post injection were determined by isotope-MS measurements. As shown in Figure 2, about 30% of the injected dose (%ID) remained in blood at day 1 post exposure, compared to only 0.2%ID of pristine SWNTs found in blood at the same post-exposure time.\[16\] The data could be approximately fitted to (In the literature $t_{1/2}$) from which $t_{1/2}$ of 15.3 hours was obtained. This is easily the longest $t_{1/2}$ time ever (in comparison with the previous record among various pharmacokinetic studies of carbon nanotubes available in the literature\[16-18\]). For example, the blood clearance was found to be very fast for both hydroxylated SWNTs and sulfonate-functionalized MWNs\[19,20\]. The half-life of surfactant-suspended pristine SWNTs in rabbits was reported to be one hour.\[21\] The previously achieved longest eliminative half-life was 3.5 hours for ammonium-functionalized SWNTs.\[22\] These results show that the blood clearance of carbon nanotubes is sensitive dependent on the nanotube surface modification, with PEGylation being the most effective in prolonging the blood-circulation time.

In light of the very recent report by Liu, et al. on noncovalently PEG-wrapped SWNTs, with $t_{1/2}$ up to 2 hours,\[23\] the covalent PEGylation is obviously a considerably more effective approach for improving the in vivo behavior of carbon nanotubes. In fact, the advantage of covalent over noncovalent in the modification of carbon nanotubes for in vivo applications is generally acknowledged in the literature.\[21,22,24,25\] For example, Chernak et al. reported the detachment of adsorbed molecules from the sidewall of SWNTs in vivo.\[25\] Sayer et al. demonstrated that covalent functionalization could reduce the toxicity of SWNTs.\[26\] According to Owen et al., covalent PEGylation is more effective in avoiding opsonization, making the nanoscale carriers less visible to phagocytic cells.\[24,25\] Similarly, poly(lactic acid) nanoparticles covalently bound with PEG chains were

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found to have longer blood circulation than nanoparticles only with surface-adsorbed PEG.\(^3\) An added benefit of covalent PEGylation is the ability to use relatively shorter PEG chains to achieve the increased avoidance characteristics of the mononuclear phagocyte system, for which the use of higher molecular weight PEG molecules is often required.\(^5\) Since long blood circulation is a pivotal factor in drug delivery, the observed stealth character in PEG-SWNTs should have far-reaching implications in the effort to use carbon nanotubes in drug-delivery systems. Here the term stealth is narrowly used to emphasize the striking blood-circulation time of PEG-SWNTs, though results already reported in the literature suggest that these PEGylated nanotubes are also protein resistant.\(^1\)

In a more detailed analysis, the experimental data in Figure 2 were better fitted by using a two-compartmental model (\(R = 0.991\)). The plasma pharmacokinetic parameters thus obtained for PEG-SWNTs were \(T_{1/2a}\) of 20 min and \(T_{1/2b}\) of 22.5 hours (Table 1). The short first-phase half-life \(T_{1/2a}\) suggests that PEG-SWNTs quickly reach the distribution balance among blood and tissues. The steady-state volume of distribution (\(V_{ss}\), Table 1) is much less than the total body water content in mice (12.5 mL)\(^6\),\(^7\) demonstrating the retention of PEG-SWNTs mostly in blood. The very long eliminative half-life \(T_{1/2b}\) and the total clearance (CL) being much lower than the hepatic blood perfusion speed (72 mL h\(^{-1}\))\(^8\)\(^9\) collectively point to a slow clearance of PEG-SWNTs from the blood.

The relatively low hepatic uptake of PEG-SWNTs was confirmed in the biodistribution study. According to the data shown in Figure 3, PEG-SWNTs were distributed throughout most organs within one hour, consistent with the observed short first-phase half-life \(T_{1/2a}\) (Table 1). No significant uptake of PEG-SWNTs could be detected in the brain, intestine, and muscle (the \(p\) value defining statistical significance >0.05). As time elapsed, gradual decreases were observed in most organs, except for liver and spleen. At day 7 post exposure, there were still considerable amounts of PEG-SWNTs in the liver (25.1 % ID or 19.1 % ID/g) and spleen (3.4 % ID or 25.9 % ID/g). The TEM analysis was used to confirm the finding of these data in the corresponding images. As shown in Figure 4, the TEM image of the specimen from the digested solution indicates clearly the presence of structurally intact SWNTs. As pointed out by a reviewer, further investigation on the biodistribution beyond 7 days is needed (though in an unrelated study with a higher initial dose of the PEG-SWNTs, preliminary results seem to suggest the detection of nanotubes in liver and to a lesser extent in spleen one month post exposure).

![Figure 2](image)

**Figure 2.** The time dependence of the SWNT concentration in blood after the mice were exposed intravenously at a dose of 2.4 mg SWNTs-equivalent/kg body weight.

![Figure 3](image)

**Figure 3.** Time-dependent biodistributions of PEG-SWNTs in mice post exposure at a dose of 2.4 mg SWNTs-equivalent/kg body weight.

### Table 1. Pharmacokinetic parameters of PEG-SWNTs in mice post intravenous exposure to a dosage of 2.4 mg SWNTs-equivalent/kg body weight.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(T_{1/2a}) (h)</td>
<td>20.0</td>
</tr>
<tr>
<td>(T_{1/2b}) (h)</td>
<td>22.5</td>
</tr>
<tr>
<td>AUC (h µg mL(^{-1}))</td>
<td>627.0</td>
</tr>
<tr>
<td>(V_{ss}) (mL)</td>
<td>3.1</td>
</tr>
<tr>
<td>CL (mL h(^{-1}))</td>
<td>0.0957</td>
</tr>
</tbody>
</table>

AUC: the area under the blood-concentration versus time curve.
The trapping in liver seems a common phenomenon for carbon nanotubes with and without functionalization, likely due primarily to the endocytosis by Kupffer cells. For example, the accumulation of tissue-functionalized carbon nanotubes in the cells was observed in mice intravenously exposed to the nanotube sample. Nevertheless, the uptake of PEG-SWNTs by the reticuloendothelial system (RES) is reduced dramatically (28%) in comparison with that of the tissue-functionalized carbon nanotubes (80%) and pristine SWNTs dispersed in Tween 80 (57%). Similar uptake reduction profiles have been observed for some nanoparticles after PEGylation.

The overall biodistribution pattern of PEG-SWNTs (Figure 3) is quite different from that of other functionalized carbon nanotubes, suggesting that chemical functionalization plays an important role in regulating the in vivo behavior of carbon nanotubes.

In our preliminary study on the tumor uptake of PEG-SWNTs, the benefit of the stealth character was already obvious. For the EMF6 model (breast cancer in BABL/c mice) and the Lewis model (lung cancer in C57BL/6 mice), the tumor uptake of intravenously administered PEG-SWNTs was 8% IDg and 9% IDg, respectively. This is considerably higher than that for SWNTs without covalent PEGylation (in the absence of any specific targeting moieties). More thorough investigations are needed to confirm these results, including a comparison of biodistributions in mice with and without the tumors.

In all in vivo experiments, no animal exhibited any signs of acute toxicological responses, even at a high exposure dose of 24 mg SWNTs equivalent/kg body weight. No animal died and no clinical abnormal signs were observed. Slow excretion of SWNTs via urine was also observed (important from the safety point of view). These observations are consistent with the report by Dumontier et al. that there is no cytotoxicity of PEG-SWNTs on primary immune cells. Further toxicological studies concerning especially chronic toxic effects of PEG-SWNTs on major target organs are planned.

In summary, the reported pharmacokinetic and biodistribution results show the distinct stealth character of PEG-SWNTs, with the observed blood-circulation time many times longer than the previous records (only for carbon nanotubes). The covalent PEGylation, which is one of the best in debundling SWNTs to obtain primarily functionalized individual nanotubes,[29,30] appears to be an effective approach in improving the in vivo behavior of carbon nanotubes. The observed higher tumor uptake, though preliminary, may have already reflected the benefit of the prolonged blood circulation of PEG-SWNTs. We believe that the development of stealth carbon nanotubes through the relatively straightforward and cost-effective PEGylation represents a significant step forward in the ongoing effort to use nanotubes for drug delivery and other biomedical systems.

Experimental Section

14C-enriched SWNTs were produced by laser ablation and purified and then functionalized with the diamine-terminated PEG oligomers (PEG1000) by following the previously reported procedure.[19,20] Briefly, the as-produced nanotube sample was treated with intermittent microwave radiation (800 W) for 20 cycles of 60 s on and 300 s off, followed by refluxing in dilute nitric acid solution (2.6%) for 12 h. Upon centrifugation (1300 g) to discard the supernatant, the solid was repeatedly washed with deionized water until neutral pH and then dried under vacuum.

The purified nanotube sample was mixed with PEG1000 and the mixture was heated to 120 °C and stirred under nitrogen protection for 3 days. The reaction mixture was cooled to room temperature and then extracted repeatedly with water to obtain the functionalized nanotube sample.[20-22] To determine the amount of PEG conjugation to SWNTs, the sample was dialyzed against fresh water to remove free PEG1000 and then analyzed quantitatively using 1H NMR and thermogravimetric analysis techniques.[20,22] The functionalized nanotubes were 25% SWNTs and 75% PEGs by weight, corresponding to one PEG1000 molecule for about 42 nanotubes. In the absence of specific experimental conditions for the shortening of SWNTs,[22] the typical length of the nanotubes was on the order of 300 nm to a micrometer or so, namely, the molecular weight of an average PEG-SWNT on the order of several million.[22] The 14C content in the sample was determined by isotope-MC (Deltaplus XP, Thermo Finnigan). For animal-exposure experiments, PEG-SWNTs were dissolved in 0.9% NaCl aqueous solution. The nanotube content in the solution was also determined by the MS method.

All animal experiments were performed in compliance with the Institutional ethics committee regulations and guidelines on animal welfare (Animal Care and Use Program Guidelines of Peking University). Male KM mice (25-30 g) were obtained from Peking University Animal Centre, Beijing (P.R. China). BABL/c mice bearing EMF6 tumor and C57BL/6 mice bearing Lewis tumor were obtained from the Cancer Institute, Chinese Academy of Medical Sciences. They were housed in metabolic cages (one mouse/cage).
and kept on a 12h light/dark cycle. Food and water were provided ad libitum. Following acclimation, mice were randomly divided into groups of 3 mice each for the in vivo experiments.

In the biodistribution study, each mouse was intravenously exposed to PEG-SWNTs at a single dose of 2.4 mg SWNTs equivalent/kg body weight (60 µg SWNTs equivalent in 200 µL). The mice were sacrificed at 1 h, 1 day, 3 days, and 7 days post exposure and their tissues, including skin, muscle (bladder), bone (shank), heart, liver, spleen, stomach, kidneys, lungs, intestine, and brain were collected. To determine the excretion pathway, urine and feces were collected daily post exposure. The samples were prepared following the previously reported procedures. The control animals were exposed to 200 µL of 0.9% NaCl aqueous solution and treated in the same way. Soicem for the 13C isotopic abundance determination were prepared in procedures generally similar to those reported previously by Oberdörster and co-workers. Briefly, tissue samples were washed with 0.9% NaCl solution and weighed after removing excess solution with filter paper. The samples from the skin, bone, urine, and feces were dried at 150 °C for 2 h and then pulverized. All other samples were mixed with 1 mL water and then homogenized for 5 min. From the homogenate about 1 mL was collected, and frozen in dry ice and acetone bath and subsequently lyophilized. Two 1.0 g aliquots from each sample (pulverized or lyophilized) were used for the 13C isotopic abundance determination. The resulting δ values were used to calculate the percentage of injected dose per gram wet tissue (μg g−1). In terms of the following equation:

\[ \% \text{ID} g^{-1} = \left( \frac{m_{\text{pag}} - m_{\text{agem}}}{m_{\text{agem}}} \right) \times 12 \times \left[ \frac{1}{13 - \delta (1000 + r) + 12} + \frac{1}{13 + \delta (1000 + r) + 12} \right] \times 100\% \]

In the equation, \( m_{\text{pag}} \) and \( m_{\text{agem}} \) are the tissue weights before and after drying, respectively. For the determination of \( m_{\text{pag}}/m_{\text{agem}} \), three healthy KM mice were sacrificed and their tissues were collected. The tissue samples were weighed and then dried under vacuum at 35 °C, during which the sample weights were checked once every 24 h until the weight change for each sample was less than 1 mg, \( m_{\text{agem}} \), which denotes carbon content in the dried sample, varied only slightly (0.48–0.53) among different tissues according to experimental results so that an average value of 0.5 was used in all calculations. \( \delta \) and \( r \) are δ-values for the test and control samples from isotope-MS measurements, respectively; \( r \) is the 13C/12C ratio (0.131737). Pet Pee De (nhenlitt), and \( \delta \) is the amount of 13C in the injection (1.58 constant in this work). The %ID g−1 is used to present the biodistribution results.

In the blood-circulation study, each mouse was exposed intravenously to PEG-SWNTs at a single dose of 2.4 mg SWNTs equivalent/kg body weight (60 µg SWNTs equivalent in 200 µL). The mice were sacrificed at 5 min, 15 min, 30 min, 1 h, 6 h, 12 h, 24 h, and 72 h post exposure and the blood samples were collected. The control animals were exposed to 200 µL of 0.9% NaCl aqueous solution and treated in the same way. The 13C contents in blood samples were similarly determined and calculated in terms of %ID g−1. The results are presented as μg SWNTs ml−1 blood (equal to %ID g−1 dose). The two-compartmental model was used to calculate the pharmacokinetic parameters for PEG-SWNTs. For comparison, the %ID of PEG-SWNTs was also calculated by first-order exponential decay (f1).

For tumor-uptake studies, B16F10 mice bearing EMFI tumor and C57BL mice bearing Lewis tumor were randomly divided into groups of 3 mice each. Each mouse was exposed intravenously to PEG-SWNTs at a single dose of 2.4 mg SWNTs equivalent/kg body weight (60 µg SWNTs equivalent in 200 µL). The mice were sacrificed on day 1 post exposure. Tumor samples were collected and prepared for the 13C isotopic abundance determination. The control animals were exposed to 200 µL of 0.9% NaCl aqueous solution and treated in the same way. The 13C contents in the tissues were determined and then the uptake was presented as percentage of injected dose per gram of wet tissue (μg g−1).

All data are presented as the mean of three Individual observations with standard error of mean. Significance has been calculated using Student’s t-test.

For TEM analysis, each mouse was exposed to PEG-SWNTs at a total dose of 2.4 mg SWNTs equivalent/kg body weight by 5 times intravenous injection of 2 mL solution once every 4 h (120 µg SWNTs/0.4 mL). Livers were collected at day 3 post exposure and then digested with 10 mL of a mixture of 65% HClO4, and 30% HNO3 (1:1 in volume) at 90 °C for 30 min. After centrifugation (10000 g for 5 min) the residue was collected and washed with deionized water. The final residue was dispersed in alcohol, from which the TEM specimen was prepared.

Keywords:
- biocompatible materials
- biodistribution
- carbon nanotubes
- stealth character

[22] The thermal method(26-28) is the functionalization of SWNTs with amino molecules presumably forms zwiterionic bonds between the amino groups and the nanotube-bound carboxylic acids (due to the oxidation of carbon defects). Though the actual mechanism and chemical structures are likely more complex and also dependent on the use of different amino molecules. For PEG-Derived specifically, the thermal method yields functionalized SWNTs that are essentially the same as those from the acylation-amination method(26) except for being experimentally more effective and stable (reproducible), thus selected in this study.

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Biodistribution of Pristine Single-Walled Carbon Nanotubes In Vivo

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The biodistribution of pristine single-walled carbon nanotubes (SWNTs) in mice was determined by using the skeleton 13C-enriched SWNTs and isotope ratio mass spectroscopy. The results suggested that the SWNTs were distributed in the entire body, with major accumulations in the liver, lungs, and spleen over an extended period of time. The specimens from the affected organ tissues were examined by using transmission electron microscopy, aimed toward an understanding of the possible uptake mechanism. The biological consequence of pristine SWNTs is obviously very different from that of their chemically modified or functionalized counterparts. The implication of such a fact is discussed.

Since the discovery, single-walled carbon nanotubes (SWNTs) have attracted tremendous research interest for their unique physicochemical properties and promises in technological applications. Recently, potential biomedical uses of carbon nanotubes, such as in drug delivery systems and for near-infrared laser absorption in selective destruction of cancer cells, have been investigated and reported. With the rapid advances in the development of carbon nanotube-based new materials and technologies, there is a growing recognition that a fundamental understanding of the pharmacological and toxicological properties of carbon nanotubes is necessary. So far, however, relevant studies are scarce, with only limited in vivo results available.1,2 For pristine SWNTs, in particular, the previously reported investigations were on pulmonary and skin toxicities only.3,4 A more systematic evaluation of the biodistribution of pristine SWNTs in vivo is urgently needed.

Quantitative studies of pristine SWNTs in vivo are relatively more difficult because of a limited selection of detection methods.5,6 In the work reported here, we took advantage of the skeleton 13C-enriched SWNTs (U13C-SWNT) to use the isotopic abundance (13C/12C) ratio determined by isotope ratio mass spectroscopy5 to track and quantify the pristine nanotubes in mice. The results offer a first glance at the more quantitative distribution pattern of pristine SWNTs (not chemically modified or functionalized) in vivo.

The U13C-SWNT sample was synthesized by laser ablation and treated to remove metal catalysts and carbonaceous impurities, as previously reported.7 The scanning TEM (Hitachi HD-2000 STEM) images suggested that the sample was generally pure and contained nanotube bundles of 10–30 nm in diameter and 2–3 μm long (Figure 1). According to results from the isotopic measurement (DELTA+ XP, Thermo Finnigan), the 13C content in the sample was 26.12% by weight. An aqueous suspension of the U13C-SWNT sample (also containing 1 wt % of Tween 80) was prepared for the animal experiments.8

All animal experiments were performed in compliance with the institutional ethics committee regulations and guidelines on animal welfare. Male KM mice (~35 g) were obtained from the Peking University Animal Center and housed in metabolic cages (one mouse/cage). Each mouse was exposed to the 13C-SWNT suspension (200 μg/200 μL) via a single tail vein injection. Urine and feces were collected daily. The mice were sacrificed at 1, 7, and 28 days post exposure, and their tissues, including skin, muscle (hind leg), bone (shank), heart, liver, spleen, stomach, kidneys, lungs, intestine, brain, spinal cord, tail, and the blood, were collected. The samples from the skin, muscle, bone, tail, urine, and feces were dried at 150 °C for 2 h and then pulverized, and other samples were prepared according to the procedure by Oberlé and et al.9 for the 13C isotopic abundance determination. The control animals were injected with an aqueous solution of 1 wt % Tween 80 (200 μL) and treated in the same way. Three mice were used for each data point. The 13C contents in the tissues were determined and calculated in terms of micrograms of 13C per gram of tissue, as reported in the literature.10 The biodistribution results are presented as percent-injected dose per gram of tissue (% ID/g). The Student t test was used for statistical analysis, and P < 0.05 was taken as the significance.

The first major difference in the biodistribution of pristine SWNTs from the chemically modified or functionalized ones is that, unlike the latter, which were cleared from the animal mostly through the renal excretory route,11 the pristine nanotubes could hardly be detected in urine and feces by using 13C isotope ratio measurements12 and TEM analyses of the samples. Therefore, the pristine SWNTs must mainly be distributed internally in different organs.

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Figure 1. A representative STEM image of the $^{13}$C-SWNT sample.

Figure 2. The biodistribution of pristine SWNTs ($^{13}$C-SWNT) in mice at different time points post exposure. Data are presented as the mean ± SD (n = 3).

The biodistribution data obtained from $^{13}$C isotopic measurements are shown in Figure 2. The SWNTs ($^{13}$C-SWNT) were apparently cleared from the blood stream quickly and distributed throughout most of the organs within 24 h, but the accumulations were primarily in the lungs, liver, and spleen. Equally significant is the fact that the nanotubes were retained in these organs at the relatively high accumulation levels over the 28 days.

The high accumulation levels in organs such as the lungs, liver, and spleen allowed direct electron microscopy analyses of residues from digested tissue solutions. In a typical experiment, mice were exposed to 600 μg of $^{13}$C-SWNT and sacrificed at 1 day post exposure. Tissues were collected and then digested with 10 mL of a mixture of 65% HClO$_4$ and 30% H$_2$O$_2$ (1:1 in volume) at 90 °C for 30 min. After centrifugation (3000 rpm x 10 min), the residue was collected and dispersed in alcohol for preparation of the TEM specimen. The residue was black-colored, consistent with the presence of carbon nanotubes that survived the aggressive digestion process. In TEM (JEM-2000CX) analyses, the characteristic structures of SWNTs could be readily identified (Figure 3), consistent with the results from the $^{13}$C isotopic measurements. The intact nanotube structures observed in TEM suggest that SWNTs are stable against the biotransformation as well as the subsequent digestion process.

TEM analyses were also used to detect the nanotubes within the tissues for exploring a possible uptake mechanism. Mice were similarly exposed to 2 mg of SWNTs and sacrificed at 1 day post exposure. Ultrathin sections were prepared from the harvested mouse liver for TEM imaging. As shown in Figure 4, the nanotubes were found to be entrapped in the phagosome of a hepatic macrophage, namely, a Kupffer cell. The observation, along with the high-level accumulations of SWNTs in organs like the liver, spleen, and lungs, suggests that the rapid uptake of the nanotubes was through the mononuclear phagocytes in the reticuloendothelial system (RES). Such an uptake mechanism involving RES is consistent with the general conception on the fate of nanoparticles in vivo but different from what was found for chemically modified SWNTs. In fact, according to Wang et al. and Singh et al., the functionalized SWNTs do not accumulate in RES organs. The difference in distributions in vivo is likely due to the different morphology and dispersion characteristics of pristine SWNTs from those of their functionalized counterparts. It may also serve as a reminder that pristine and functionalized carbon nanotubes should be treated differently in terms of their biological consequences.

On the per-organ basis (Table 1), liver was, by far, dominating the uptake of pristine SWNTs. However, the lungs were not
so much behind. The accumulation of intravenously administrated SWNTs in the lungs is probably not surprising, as reports of similar observations for other materials, such as polymer microspheres, are available in the literature.\(^{20,21}\) In these organs of major accumulations, the changes in uptake over time (shown in Table 1 in % ID/organ) were somewhat different. While the level in the liver was relatively constant, there was a gradual decrease in the lungs from 15% to 9.4% in 28 days. Two possible pathways may be considered for the clearance of SWNTs from the lungs.\(^{20}\) One is that the nanotubes are secreted by the alveolar macrophage as mucus through mucociliary transport to leave the lungs. The other is that the interstitial SWNTs are transferred through the lymph nodes and finally into the spleen. This seems consistent with the observed gradual increase in the uptake by the spleen over the same time period (Table 1).

Pristine SWNTs were also distributed in many other organs and tissues (Figure 2). Most noticeable and interesting is the clearly detectable (1.0–3.3% ID/g) accumulation of the nanotubes in the brain over the 28 days of the experimental period, suggesting that the nanotubes could overcome the blood brain barrier to enter into the brain (possibly taken up by the brain capillary endothelial cells under the assistance of polymer-coated receptor-mediated phagocytosis).\(^{22}\) This first of the kind of observation is still preliminary, and further experimental verification and mechanistic elucidation are required.

During the experimental period, no animal exhibited any signs of acute toxicity responses, even at a high exposure dose of 2 mg of \(^{14}C\)-SWNT per mouse. No animals died during the 4 week test period, and no clinical signs of abnormalities were observed. The clearance of the nanotubes from most organs was generally slow, with an estimated average total clearance rate of less than 1% per day. Further toxicological studies, especially the chronic toxic effects of SWNTs on major target organs, are needed.

In summary, the results reported here show a distinct profile of in vivo biodistribution for pristine SWNTs, with little excretion via urine and feces and major accumulations in the liver, lungs, and spleen over an extended period of time, thus suggesting that these materials should be treated differently from their chemically modified or functionalized counterparts in terms of their biological consequences. The results also suggest that the isotope ratio mass spectrometry, coupled with \(^{14}C\)-enriched samples, is an effective technique for biodistribution studies of nanomaterials. In further investigations, a better understanding of the uptake mechanism will be pursued, and potential uses of the biodistribution (such as for targeted drug delivery) will be explored.

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nanoscience and nanotechnology. The financial support from the China Natural Science Foundation (significant Project No. 10490180 and special Project No. 90466024-5) and the China Ministry of Science and Technology(973 Project No. 2006CB705004) to the Peking University group and from the U.S. National Science Foundation to Y.-P.S. is gratefully acknowledged.

References and Notes

(9) During the preparation of this manuscript, Cherniakov et al. 10 reported the use of nanotube fluorescence to track surfactan-dispersed pristine HPC-SWNTs in solution, in which significant concentrations of monomers were found only in the liver.

(14) The detection limit for the CO2/CO ratio is about 1 ppm. If the injected SWNTs (8H-CNT) were excreted via urine and feces already throughout the 36 days, the detected CO2/CO ratio in the urine would be 5 times of the detection limit, while the ratio in the feces would be close to the limit. Therefore, the monomers were definitely not excreted via urine but could, in principle, be excreted in marginally detectable amount via feces.
Carbon Dots for Multiphoton Bioimaging
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Two-photon fluorescence materials have attracted much recent attention for their many promising applications, especially in the growing field of biomedical imaging.1-3 Among the best performing two-photon fluorescent materials are semiconductor quantum dots such as CdSe and related core−shell nanoparticles.4-8 These quantum dots have been demonstrated in various optical imaging experiments in vitro and in vivo.9-10 At the same time, however, heavy metals as the essential elements in available high-performance semiconductor quantum dots have prompted serious health and environmental concerns in the community and beyond. Therefore, the search for benign alternatives has become increasingly important and urgent. Recently, we found and reported11 that nanosized pure carbon particles may be surface-passivated to exhibit bright photoluminescence in the visible. These photoluminescent carbon dots (C-Dots, Figure 1a) are of two distinctive features: one is that the underlying carbon particles are very small (sub-10 nm); and the other is that the particle surface is passivated by organic or other molecules via either covalent linkages or chemical adsorption.12 Mechanically, the carbon-based photoluminescence has been attributed to passivated defects on the carbon particle surface acting as excitation energy traps.13,14 Here we report that the C-Dots also exhibit strong luminescence with two-photon excitation in the near-infrared. The estimated two-photon absorption cross-sections of the C-Dots are comparable to those of available high-performance semiconductor quantum dots. In addition, the two-photon luminescence microscopy imaging of the C-Dots internalized in human cancer cells is demonstrated.

The C-Dots were prepared as previously reported.11 Poly(propyleneimine-co-ethylamine) (PPEI-Et, with Et fraction ~20%) was used as the surface passivation agent. The C-Dots thus prepared were readily soluble in water to yield a colored aqueous solution. Shown in Figure 1b is a representative AFM image of the C-Dots on mica substrate, from which feature sizes of generally less than 5 nm are identified.

The specimen for optical microscopy was prepared by first drooping a small aliquot of the aqueous solution on cover glass and then evaporating the water. A Leica confocal fluorescence microscope equipped with an argon ion laser and a femtosecond pulsed Ti:sapphire laser was used. The C-Dots were found to be strongly emissive in the visible with either the argon ion laser excitation (458 nm) or the femtosecond pulsed laser for two-photon excitation in the near-infrared (800 nm). As compared in Figure 2, the one- and two-photon luminescence images for the same scanning area match well. The C-Dots were photostable under the two-photon imaging conditions (upon repeated 100 mW excitation equivalent to generating the image in Figure 2b for at least 3000 times, so meaningful changes in emission intensities).

The same microscope setup was used to measure the two-photon spectra. For the same specimen (C-Dots deposited on cover glass), the observed spectra vary slightly from spot to spot, reflecting the inhomogeneous nature of the sample. A representative two-photon luminescence spectrum of average C-Dots is shown in Figure 3.

Figure 1. (a) The C-Dot structure; (b) AFM topography image of C-Dots on mica substrate, with the height profile along the line in the image.

Figure 2. Luminescence images (all scale bars 20 μm) of the C-Dots with (a) argon ion laser excitation at 458 nm and (b) femtosecond pulsed laser excitation at 800 nm; (c) is an overlay of (a) and (b).

Figure 3. (Left) The one-photon (Δ, 458 nm excitation) and two-photon (O, 800 nm excitation) luminescence spectra of the C-Dots on glass substrate (prepared with infinite dilution, and optical spot diameter ~500 μm, covering multiple dots immobilized on the substrate) are compared with solution-phase absorption (ABS) and luminescence (solid line, 400 nm excitation) spectra. (Right) The quadratic relationship of the observed two-photon luminescent intensity of the C-Dots on glass substrate with the excitation laser power at 800 nm (P800, as measured at the focal plane).

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The two-photon absorption cross-section \( \sigma_{\text{2P}} \) of C-Dots was estimated by determining the two-photon luminescence intensities of the specimen and a reference under the same experimental conditions: \( \sigma(\lambda) = c_{\text{ref}}(\lambda) \left( \frac{F(\lambda)_{\text{spec}}}{F(\lambda)_{\text{ref}}} \right)^{2} \left( \Phi_{\text{spec}} \right) \), where \( F(\lambda) \) represents average luminescence photon fluxes (or experimentally observed emission intensities), \( \Phi \) is luminescence quantum yields, and the subscript ref denotes values for the reference compound. By using rhodamine B as the reference,\(^\text{19} \) the two-photon absorption cross-sections of C-Dots at different excitation wavelengths were calculated from the experimental results. At 800 nm, the average \( c_{\text{2P}} \) value for the C-Dots was 19.000 ± 3000 GM (Goeppert-Mayer unit, with 1 GM = 10^{-60} \text{cm}^{4} \text{s}^{-1} \text{cm}^{-2} \text{eV}^{-1} \text{photons})

It makes the C-Dots comparable and high-performance to other two-photon luminescent nanomaterials.\(^\text{19,40} \) For example, the two-photon absorption cross-section of CdSe quantum dots at 800 nm varies in the range of 780–10 300 GM, depending on the particle size.\(^\text{4} \) For CdSe/ZnS core–shell quantum dots (fluorescence at 665 nm), the two-photon absorption cross-section was estimated to be on the order of 50 000 GM.

In an exploratory experiment to demonstrate the potential of C-Dots for cell imaging with two-photon luminescence microscopy, human breast cancer MCF-7 cells were cultured in terms of the established protocol.\(^\text{20} \) Upon incubation with the C-Dots in an aqueous buffer at 37 °C, the MCF-7 cells became brightly illuminated when imaged on the fluorescence microscope with excitation by 980 nm laser pulse. As shown in Figure 4, the C-Dots were able to label both the cell membrane and the cytoplasm of MCF-7 cells without soaking the nucleus in a significant fashion. The translocation of the C-Dots from outside the cell membrane into the cytoplasm is temperature-dependent, with no meaningful C-Dots internalization observed at 4 °C. While endocytosis is likely, at understanding the internalization mechanism still requires more investigations. In addition, a better accumulation of C-Dots in the cell (even in the nucleus) may be achieved by C-Dots coupled with membrane translocation peptides such as TAT (a human immunodeficiency virus-derived protein), which facilitates the translocation of the tissue by overcoming the cellular membrane barrier and enhances the intracellular labeling efficiency.\(^\text{12,19} \) This is being pursued along with a comparison between one- and two-photon luminescence imaging of cells labeled with C-Dots.

In summary, C-Dots are strongly two-photon active, with the pulsed laser excitation in the near-infrared to result in bright luminescence in the visible. The estimated two-photon absorption cross-sections of the C-Dots are comparable to those of the best-performing semiconductor quantum dots or core–shell nanomaterials already reported in the literature. Available results from exploratory experiments of luminescence imaging in vitro suggest that the

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**Figure 4.** Representative two-photon luminescence image (800 nm excitation) of human breast cancer MCF-7 cells with internalized C-Dots. More details on the cell experiment: MCF-7 cells (approximately 5 × 10^5) were seeded in each well of a four-chambered LabTek collagen coated cover glass system (Irvine Scientific) and cultured at 37 °C. All cells were incubated until approximately 50% confluence was reached. Separately, an aqueous solution of the C-Dots (0.9 mg/mL) was passed through a 0.2 μm sterile filter membrane (Millipore, Bedford, Mass). The fiber bundle (20–40 μm) was mixed with the cell medium (500 μL) and then added to three wells of the glass slide chamber (the fourth well served as a control) in which the MCF-7 cells were grown. After incubation for 2 h, the MCF-7 cells were washed three times with PBS (100 μL each time) and kept in PBS for the optical imaging.

C-Dots are internalized into the human breast cancer cells likely through endocytosis, demonstrating the potential of the C-Dots in cell imaging with two-photon luminescence microscopy.

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**Supporting Information Available:** Complete ref 11 and histograms of luminescence intensities in the image. This material is available free of charge via the Internet at http://pubs.acs.org.

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Diameter-Selective Fractionation of HiPco Single-Walled Carbon Nanotubes in Repeated Functionalization Reactions

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The functionalization reaction of the diamine-terminated oligomeric poly(ethylene glycol) with a purified HiPco single-walled carbon nanotube sample was repeated multiple times to yield the corresponding number of soluble fractions and the final insoluble residue. According to results from the characterizations with various instrumental techniques, these soluble fractions and the final residue all have different nanotube diameter distributions from that found in the starting sample. A general conclusion is that the functionalization and solubilization are selective toward smaller diameter nanotubes, regardless of their being semiconducting or metallic. Mechanistic implications, especially with respect to the post-production separation in terms of the nanotube metalliclicity, are discussed.

Introduction

A single-walled carbon nanotube (SWNT) is characterized by a pair of chiral indices (n, m), which determine the tube diameter d in terms of the relationship as follows:1

\[ d = \frac{\sqrt{n^2 + m^2 + \sqrt{n^2 + m^2}}}{\sqrt{5}} \]

where \( a_{cc} \) is the nearest neighbor carbon–carbon distance. The indices also define the metallicity of the nanotube: a semiconducting SWNT with \( \text{mod} \ (\{n,m\}, 3) = 0 \) and a metallic SWNT with \( \text{mod} \ (\{n,m\}, 3) = 1 \) or 2. Various production methods for SWNTs generally yield mixtures of different chiralities. In fact, it is well known that the SWNTs from the high-pressure CO disproportionation (HiPco) process are of a particularly broad distribution in tube diameters (\(~0.7-1.3 \text{ nm}\)).

There have been significant efforts on post-production separation of SWNTs aimed toward their ultimate "purification" in terms of chiralities, with especially heavy emphasis on harvesting semiconducting or metallic-enriched SWNTs.9-6 Interestingly, with only a few exceptions,7-9 the repeated post-production separation experiments have been centered on HiPco-SWNTs.9-10 The separation effort exploits potentially selective interactions of functional molecules with semiconducting or metallic SWNTs. For example, Tour, Smalley, and co-workers reported that some highly reactive species such as diazonium salts were preferentially added to the sidewalls of metallic HiPco-SWNTs.11 Similar preferential additions of nitrosoxide ions and fluorene gas were reported recently by others.12-13 Papadimitrakopoulos and co-workers initiated the approach of using long-chain molecules bearing amine moieties to remove some semiconducting HiPco-SWNTs from the purified mixture.14-16 Where the basis of separation was apparently the selectivity of the functionalization agent octadecylamine (ODA) toward semiconducting nanotubes for their solubilization. Maeda et al.17 reported recently that an opposite selectivity could be achieved with the use of smaller amine molecules such as octylamine and propylamine, namely that metallic instead of semiconducting nanotubes were enriched in the supernatant. On the other hand, the reactivity and interactions of a nanotube with other species are known to be dependent on the tube diameter (or the graphene armchair corresponding to pyramidalization and π-π orbital misalignment).17,18 In fact, there is an inverse relationship between the nanotube reactivity and diameter, with the smaller SWNTs being more reactive. Therefore, a significant issue in the post-production separation of SWNTs is the likely interplay between the selectivity toward the tube diameter and the selectivity toward the semiconducting or metallic characteristics.

According to reagents on the reactivity-based post-production separation, the nitrosonium ion attack prefers smaller diameter metallic SWNTs,19 while the ozonolysis reaction is selective toward smaller diameter SWNTs regardless of metalliclicity.19 Also, the selective interactions of ODA molecules with semiconducting SWNTs17 were found to be more pronounced with the nanotubes of smaller diameters.20

We reported previously an investigation on repeated functionalization reactions to sequentially extract soluble fractions from arc-discharge-produced SWNTs.20 Despite the intrinsically narrow diameter distribution in the starting nanotube sample, the fractionation results with the use of diamine-terminated oligomeric poly(ethylene glycol) (PEG,1,000) as functionalization agent suggest a clear preference of the functionalization reaction toward smaller diameter SWNTs. In the work presented here, the same approach and similar experimental conditions were applied to the repeated functionalization reactions with HiPco-SWNTs, in which the diameter-selective fractionation in the solubilization became more evident. The results from the characterization of the fractionated samples are described, and their implication to the effort on using similar functionalization reactions for post-production separation of semiconducting and metallic SWNTs is discussed.
HiPco Single-Walled Carbon Nanotubes

Experimental Section

Materials. Diamine-terminated oligomeric poly(ethylene glycol) (H₂N(CH₂)₆CH₂(CH₂O(CH₂)₆CH₂)₆N₂H) with a ~ 35, or PEG(3500) and sodium dodecyl sulfate (SDS) were purchased from Aldrich, and deuterated chloroform and D₂O were purchased from Cambridge Isotope Laboratories. Dialysis tubing (cellulose membranes) with a molecular weight cutoff of ~13,000 was obtained from Sigma-Aldrich.

The HiPco-SWNT sample was supplied by Carbon Nanotechnologies, Inc. and was purified by using a procedure adapted from what is available in the literature.3 In the purification, the as-supplied nanotube sample (500 mg) was treated with intermittent microwave (800 W) for 20 cycles of 60 s on and 300 s off, followed by refluxing in dilute nitric acid solution (2.6 M, 250 mL) for 12 h. Upon centrifugation (3300 g) to discard the supernatant, the solid was repeatedly washed with deionized water until neutral pH was obtained, and then the sample was dried under vacuum to yield the purified HiPco-SWNT sample (~250 mg).

Measurements. Several centrifuge machines were used for low- (Fischer Scientific, Centrifuge Model 228) and high-speed centrifugation (Beckman-Coulter Optima L90K ultracentrifuge with a type 90 Ti fixed-angle rotor). UV/vis/near-IR absorption spectra were recorded on a Shimadzu UV3500 spectrophotometer. Raman spectra were obtained on a Renishaw Raman spectrometer equipped with a 50 mW diode laser source for 785 nm excitation, and a Jobin-Yvon T64000 spectrometer equipped with a triple monochromator, a research-grade Olympus BX-41 microscope, a liquid-nitrogen-cooled symphony detector, and two excitation sources (Melles-Griot 35 mW He:Ne laser for 632 nm and Spectra-Physics 40 mW argon-ion laser for 514 nm excitations). Thermogravimetric analysis (TGA) experiments were performed on a Mettler-Toledo TGA/SDTA851e system. Transmission electron microscopy (TEM) digital images were obtained on Hitachi HF-2000 TEM and Hitachi HD-2000 STEM/TEM systems.

Functionalization Reactions.23,24 In a typical experiment, a mixture of purified HiPco-SWNTs (225 mg) and PEG(3500) (3.8 g) was heated to 120 °C and stirred under nitrogen protection for 4 days. After the reaction, the mixture was cooled to room temperature and then extracted repeatedly with water for the soluble fraction. Each extraction involved the addition of distilled water (15 mL) and then centrifuging at 1380 g to collect the colored supernatant. Typically five repeats were necessary until the supernatant became colorless. The combined soluble fraction was cleaned via dialysis (membrane molecular weight cutoff of ~12,000) against fresh distilled water for 3 days, and then evaporated to remove water. The insoluble residue after the repeated extractions was dried under vacuum for the next round of functionalization reaction.

In each subsequent functionalization reaction, the same PEG(3500)/nanotube weight ratio of 17:1 was used, and so were the reaction conditions. The reaction mixture was extracted in the same procedure to separate the soluble fraction from the insoluble residue. All soluble fractions from the repeated functionalization reactions and the final insoluble residue were dried for their various characterization experiments.

Results and Discussion

The functionalization of HiPco-SWNTs with PEG(3500) was under thermal reaction conditions.23,24,25 Mechanistically, there is presumably the formation of a zwiterionic bond between the amino group in PEG(3500) and the nanotube-bound carboxylic acid (due to the oxidation of carbon nanotube defect), though noncovalent direct adsorption of PEG(3500) on the nanotube graphitic surface may also play a significant role in the stabilization of the nanotube.3 Nevertheless, the functionalization of HiPco-SWNTs with PEG(3500) for the nanotube stabilization was relatively robust. The solid-state samples for the soluble fractions from the repeated functionalization reactions could readily be dissolved in water or various polar organic solvents. These aqueous or organic solutions were stable over an extended period of time (at least several months), again demonstrating the robustness of the functionalization and stabilization.

Fractionation. A total of five soluble fractions were collected from the five repeats of functionalization reactions. The amount of nanotubes solubilized in each functionalization reaction varied, with the first and second reactions solubilizing 23% and 28% of the starting HiPco-SWNTs, respectively (Table 1). By the fifth reaction, the amount of nanotubes solubilized decreased to 2% of the starting HiPco-SWNTs (Table 1), suggesting no need for further repeats.

The nanotube contents in the soluble fractions and the final residue (listed in Table 1) were determined by using TGA. Shown in Figure 1 are typical TGA traces for the samples. At a heating rate of 10 °C/min in nitrogen atmosphere, the PEG(3500) functional groups could be removed completely at about 450 °C,25,26 while the defunctionalized HiPco-SWNTs remained stable. Quantitatively, the amount of recovered nanotubes from the five soluble fractions and the final residue totaled 235 mg (about 71% in the soluble fractions and 29% in the final residue). In reasonable agreement with the amount of starting HiPco-SWNTs (225 mg). As detailed in Table 1, the nanotube contents in the soluble fractions decreased significantly in subsequent functionalization reactions after the first two repeats, indicating the inhomogeneous nature of the starting purified HiPco-SWNT sample with respect to functionalization and solubilization.
The different fractions share some similar material properties. For example, TEM techniques were used to characterize the soluble fractions and the final residue. In the specimen preparation, a drop of diluted aqueous solution of the soluble fraction (or aqueous suspension for the final residue) was placed onto a carbon or holey carbon-coated copper grid, followed by solvent evaporation. The TEM images of the soluble fractions show no meaningful differences, as compared in Figure 2. For the final residue, the TEM image suggests that the nanotubes are in larger bundles than those in the soluble fractions, as one might expect.

The soluble fractions were also evaluated by using 1H NMR. At a relatively dilute concentration of 10 mg/mL in CDCl₃, the observed spectra of the fractions appear similar, each with a broad signal centered at ~3.6 ppm (Supporting Information). This is readily assigned to the ethylene protons in the PEG₂₀₀₀ functional groups. The broadness of the peak is consistent with the functional groups being attached to SWNTs, which are species with large molecular weights and low mobilities.

Diameter Selectivity. The optical spectroscopy results of the soluble fractions and the final residue suggest significant diameter preference in the repeated functionalization reactions, resulting in different diameter distributions of HiPco-SWNTs in the various fractions.

The solution-phase optical absorption spectra of the soluble fractions (except for the fifth fraction due to limited sample quantity) were measured in D₂O to avoid spectral interference in the near-IR region. The solutions were prepared by directly dissolving the solid samples into D₂O. Similarly, the starting purified HiPco-SWNT sample and the final residue were suspended in D₂O with the assistance of SDS (0.5 mg/mL), which is a surfactant widely used in the literature for optical spectroscopy of SWNTs. These solutions and suspensions in D₂O (all with 2 mg/mL nanotube-equivalent concentrations) were centrifuged at 137 000g for 2 h before the optical spectral measurements. As shown in Figure 3, the spectrum of the starting purified sample is resolved with spectral features typical of HiPco-SWNTs after similar purification treatment. The peak features at 900–1600 nm and 550–900 nm are commonly assigned to groups of electronic transitions corresponding to the first and second pairs of von Hove singularities in the density of states (DOS) for various semiconducting SWNTs (denoted as S₁ and S₂), respectively, and these at 400–650 nm corresponding to first transitions of various metallic SWNTs (denoted as M₁).

The absorption spectra of the solubilized samples remain similarly resolved with S₁, S₂, and M₁ peak features in their respective spectral ranges (Figure 3), suggesting that the nanotube electronic structures are retained in the functionalization with PEG₂₀₀₀. This is consistent with previously reported results on the PEG₂₀₀₀ functionalization of SWNTs from other production methods.

A closer examination of the absorption spectra reveals significant and systematic changes in the detailed spectral peak features from fraction to fraction, reflecting the underlying variations in diameter distributions of the HiPco-SWNTs. For the S₁ bands, more features of the first soluble fraction are at shorter wavelengths (roughly below 1200 nm) in comparison with those of the starting purified sample. In the later soluble fractions, however, more significant spectral features are in the longer wavelength region (especially above 1200 nm). The changes in S₂ bands are in a similar trend, with those of the first soluble fraction featured more in the 650–750 nm region, while those of later fractions are more in the 750–900 nm region (Figure 3). It is known that electronic transition energies of SWNTs are inversely related to the nanotube diameters. Thus, the absorption results suggest that the smaller diameter semiconducting SWNTs were preferentially solubilized in the earlier fractions.

The diameter-selective fractionation in the repeated functionalization reactions is more evident in the results of resonance Raman spectroscopy. The Raman measurements were performed at multiple excitation wavelengths of 785 nm (1.58 eV), 632 nm (1.96 eV), and 514 nm (2.41 eV). In order to avoid the
known luminescence interference with functionalized nanotube samples,\textsuperscript{16} the soluble fractions and the final residue were thermally defunctionalized (heated to 600 °C and kept for 2 h in an inert atmosphere) before the Raman characterization.

For the starting purified sample, the Raman spectrum at 785 nm excitation exhibits typical features of the G-band at 1592 cm\textsuperscript{-1}, D-band at 1339 cm\textsuperscript{-1}, and radial breathing modes (RBMs) in the 200–300 cm\textsuperscript{-1} region (Figure 4), in agreement with those reported previously.\textsuperscript{10,12} Here the 785 nm excitation is in resonance with mostly semiconducting HiPco-SWNTs of various diameters but with few of their metallic counterparts.\textsuperscript{15} Also shown in Figure 4 are Raman spectra of the soluble fractions and the final residue with the same 785 nm excitation. The G-band features are similar among all these samples, with the bands being relatively narrow and symmetrical. However, there are systematic changes in the RBM region, with the spectra of the earlier fractions enriched with higher frequency features (corresponding to smaller diameter semiconducting SWNTs) and with those of the later fractions and the final residue with lower frequency features (corresponding to larger diameter semiconducting SWNTs). For example, the spectrum of the first soluble fraction shows the 264 cm\textsuperscript{-1} peak higher in intensity than peaks in the 188–216 cm\textsuperscript{-1} and 217–242 cm\textsuperscript{-1} regions, but the spectrum of the fifth soluble fraction exhibits a nearly opposite peak intensity pattern (those of the other fractions are apparently intermediate between the two) (Figure 4). In the spectrum of the final residue, only peaks at 189 and 206 cm\textsuperscript{-1} could be observed. It is known that the RBM peak frequency (in cm\textsuperscript{-1}) is inversely related to the corresponding SWNT diameter (d, nm) in terms of the equation d = 22.5/f + 12.5 (see also Supporting Information).\textsuperscript{12} Thus, the initial functionalization reaction in multiple repeats was obviously selective toward the smaller diameter nanotubes among the population of semiconducting HiPco-SWNTs.

The Raman spectra were also measured with 632 and 514 nm excitations (coupled with the use of a triple monochromator) for a closer examination of the low-frequency region, despite the fact that different HiPco-SWNTs (from those with 785 nm excitation) are in resonance with these excitation wavelengths. The RBM features in the spectra exhibit systematic changes in a trend similar to that found with the 785 nm excitation. Generally the earlier fractions are with more intense higher-frequency RBM features, and the later fractions and the final residue are with more intense lower-frequency RBM features.
G-bond results with 632 and 514 nm excitations are consistent with the enrichment of larger diameter HiPco-SWNTs (indicating semiconducting and metallic ones, respectively) in the later soluble fractions and the final residue. Overall, the optical absorption spectra and resonance Raman results with three different excitation wavelengths appearly suggest that the major functionalization reaction of PEG-PEG-A with the purified HiPco-SWNT sample is selective toward these nanotubes of smaller diameters, regardless of their being semiconducting or metallic. This may have significant implications to the effort on the post-production separation of semiconducting and metallic SWNTs.4.10

Mechanical and Other Implications. A characteristic feature of as-supplied or purified HiPco-SWNTs is the broad distribution of nanotubes of different diameters. Therefore, it is hardly surprising that the diameter selectivity becomes an issue in various functionalization reactions. For the thermal reaction conditions used in this study, the preference of PEG-PEG-A molecules toward smaller diameter HiPco-SWNTs is probably associated with their direct adsorption onto the nanotube surface. The role of such noncovalent but specific interactions in the solubilization of carbon nanotubes has been suggested for other long-chain functionalization agents with primary amino moieties.7.18 As for the selectivity toward smaller diameter HiPco-SWNTs, the specific interactions responsible for the direct adsorption may be mechanistically similar to those found in the addition of highly reactive species to the nanotube sidewalls.10,12,13

The diameter selectivity in functionalization reactions (involving the adsorption) could potentially alter the population balance between semiconducting and metallic nanotubes if the two nanotube types were of different diameter distributions, which causes a marked increase in the average size of the nanotubes in one over the other in various reaction mixtures or fractions. Hypothetically, should the starting sample be populated with more smaller diameter semiconducting nanotubes than their metallic counterparts, the preferential solubilization of the former would obviously leave more metallic nanotubes in the insoluble residue, and vice versa. Therefore, it might be possible, at least in principle, that the interplay between diameter and metallicity fluctuations in the functionalization and solubilization of HiPco-SWNTs with long-chain amino compounds like PEG-PEG-A and ODA could be driven primarily by the diameter selectivity. This is an interesting topic that deserves further investigations.

In summary, the repeated functionalization reactions of PEG-PEG-A molecules with purified HiPco-SWNTs resulted in multiple soluble fractions and the final insoluble residue. These fractions have different nanotube diameter distributions from that found in the starting purified HiPco-SWNT sample. A general conclusion is that the first nanotubes solubilized are smaller diameter ones, regardless of their being semiconducting or metallic. The results reported here and those already in the literature suggest that because of the intrinsically broad diameter distribution in HiPco-SWNTs, diameter selectivity plays an important role in the reactions or specific interactions designed or exploited for the post-production separation of the nanotubes into semiconducting and metallic enriched fractions. Further experimental investigations on the existing and other amino-functionalization or dispersion agents are required for a better understanding of the various selectivities.

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Supporting Information Available: ‘H NMR spectra of the soluble fractions and a showing calculated absolute diameters from Raman RBK peaks. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes


HiPro Single-Walled Carbon Nanotubes


Quantum-Sized Carbon Dots for Bright and Colorful Photoluminescence

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Fluorescent semiconductor quantum dots have generated much excitement for a wide variety of promising applications, especially those in biology and medicine. For both in vitro and in vivo uses, however, the known toxicity and potential environmental hazard associated with many of these materials may represent serious limitations. Therefore, the search for benign nanomaterials of similar optical properties continues. For quantum-sized silicon, the discovery of Bras and co-workers on the strong luminescence in surface-oxidized nanocrystals has attracted extensive investigations of silicon nanoparticles and nanowires. For example, silicon nanoparticles capped with water-soluble polymers, that are compatible with physiological media, have been studied for the luminescence labeling of cells. Here we report a new finding on the quantum-sized carbon analogues, namely, that nanoscale carbon particles ("carbon dots") upon simple surface passivation are also strongly photoluminescent in both solution and the solid state with spectral features and properties comparable to those of surface-oxidized silicon nanoparticles. These strongly emissive carbon dots may find applications similar to or beyond those of their widely pursued silicon counterparts.

The carbon dots were produced via laser ablation of a carbon target in the presence of water vapor with argon as carrier gas. The carbon target was prepared by hot-pressing a mixture of graphite powder and cement, followed by stepwise burning, curing, and annealing in argon flow. A Q-switched Nd:YAG laser (1064 nm, 10 Hz) was used for the ablation, during which the carbon target was in a flow of argon gas carrying water vapor (through a water bubbler) at 908 °C and 75 kPa. The as-produced sample, according to electron microscopy analyses, was dominated by nanoscale carbon particles in aggregates of various sizes. There was no detectable photoluminescence from the sample and its aqueous suspension. The sample was reacted in an aqueous nitric acid solution (up to 2.6 M) with refluxing for up to 12 h. The treated sample still exhibited no detectable photoluminescence. However, upon the surface passivation by staining simple organic species to the acid-treated carbon particles (Scheme 1), bright luminescence emissions were observed (Figure 1).

Many organic molecules could serve as the purpose of surface passivation. For example, diamineterminated oligomeric polyethylene glycol) H2NCH2CH2(CH2)nCH2CH2NH2 (average n ~ 35, PEG(1000)) was used to react with the carbon nanoparticles (Scheme 1). In a typical reaction, PEG(1000) (300 mg, 0.13 mmol) was mixed with an acid-treated particle sample, and the mixture was heated to 120 °C for 72 h. After the reaction, the mixture was cooled to room temperature and dispersed in water, followed by centrifuging (~1400g) for 30 min. The colored but homogeneous supernatant contained the carbon dots with PEG(1000) species attached to the surface (Scheme 1). A small fraction of the supernatant was diluted for the preparation of microscopy specimens, deposited on a carbon-coated copper grid for scanning transmission electron microscopy (STEM) and on a mica surface for atomic force microscopy (AFM). The results suggest that these carbon dots are around 5 nm in diameter (Figure 2).

Other molecules or polymers, such as poly(propargyl) and poly(ethyleneimine-co-ethylenimine) (PPEI-EE), could also be used in largely the same reaction procedure for surface passivation to achieve similar results (Figure 2).

The passivated carbon dots with organic moieties attached to the surface are strongly photoluminescent both in the solution-like...
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Figure 3. The absorption (ABS) and luminescence emission spectra (with progressively longer excitation wavelengths from 400 nm on the left in 20 nm increments) of PPEI carbon dots in an aqueous solution. The emission spectral intensities are normalized to quantum yields (normalized to spectral peaks in the inset).

suspension and in the solid state, and the emissions cover the visible wavelength range and extend into the near-infrared (Figure 1). It should be pointed out that the organic and polymeric passivation agents (PEG,PDMS and PPEI-ED) could contain no visible or near-UV chromophores and therefore are obviously nonemissive at visible wavelengths. The observed bright and colorful luminescence emissions must be due to the surface-passivated carbon dots. As shown in Figure 3, the photoluminescence spectra of the carbon dots are generally broad and dependent on excitation wavelengths (Figure 1), which as in their silicon counterparts may reflect not only effects from particles of different sizes in the sample but also a distribution of different emissive sites on each passivated carbon dot.

The brightness of the photoluminescence is reflected in the high emission quantum yields. At 400 nm excitation, the observed quantum yields were from about 4% to more than 70%, where the variation probably depended on the effectiveness of the reaction for surface passivation. For example, when a sample of PEGPDMS or PPEI-ED attached carbon dots was found to have the emission quantum yield at the lower end of the range, the sample could be reactivated again with PEG-PS or PPEI-ED under the same reaction conditions (thus improved surface passivation) to become more emissive with a higher quantum yield. Further effort is required to understand and better control the parameters dominating the particle surface passivation and resulting properties for carbon dots of brighter photoluminescence. Generally speaking, however, the present observed luminescence emissions of the carbon dots are comparable with those of traditionally prepared and passivated silicon nanocrystals.

The photoluminescence of the carbon dots is stable with respect to photostability, exhibiting no meaningful reduction in the observed intensities in the experiment of continuously repeating excitations for several hours. Unlike many other fluorescent nanoparticles, there is no bleaching in the luminescence emissions of the carbon dots according to laser-scanning confocal microscopy analyses (commercially available gold nanorods is control under the same measurement conditions).

Mechanistically, the photoluminescence from carbon dots may be attributed to the presence of surface energy traps that become emissive upon stabilization as a result of the surface passivation. The requirement for surface passivation to become photoluminescent is apparently shared by the carbon dots and the silicon nanocrystals, for which a widely accepted mechanism for luminescence emission is the radiative recombination of excitons. For the photoluminescent carbon dots reported here, however, there must be a quantum confinement of energetic energy traps to the particle surface, namely, that a large surface-to-volume ratio in a particle is necessary in order for the particle upon surface passivation to exhibit strong photoluminescence. There has already been experimental evidence supporting such a semiclassical argument. Larger carbon particles (30–50 nm in average diameter, for example) with the same surface passivation were found to be much less luminescent. Conversely, it might be expected that PEG-PS attached photoluminescence quantum yields be achieved in smaller carbon dots with the same or similar surface morphology and passivation.

As in their silicon counterparts, the carbon dots may, in principle, be separated or manipulated such that some of the inhomogeneity in photoluminescence is removed. On the other hand, the inhomogeneity may be exploited in the use of the surface-passivated carbon dots for optical labeling to allow the selection of different emission colors with different excitation wavelengths (in confocal microscopy, for example). The versatile surface functionalities as required for passivation will also be very useful for the carbon dots in bioimaging applications. The tracer, such as PEGED, are not only aqueous compatible but also readily conjugated with antibodies or other bioactive molecules. Preliminary results on the optical imaging of biological species with the emissive carbon dots are provided in the Supporting Information.

Supporting Information Available: Additional characterization results, and preliminary results on the optical imaging of biological species. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) The linkages for the attachment might be similar to those found in the functionalization of carbon nanotubes at surface defect sites (see ref 10, for example).
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Solubilization of boron nitride nanotubes

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A successful attempt in the functionalization and solubilization of boron nitride nanotubes is reported, and a functionalization mechanism based on interactions of amino functional groups with nanotube surface borons is proposed. There has been much recent interest in boron nitride nanotubes (BNNTs) for their electronic,1-3 thermal,4-6 and mechanical properties,7-9 which are in many cases different from and/or superior to those of carbon nanotubes. For example, BNNTs are semiconductors with a wide band-gap (4.5 eV) independent of the nanotube chirality or morphology.1-3 The thermal conductivity, oxidation resistivity, thermal and chemical stability, and yield resistance of BNNTs are higher than those of their carbon analogs. Among widely discussed unique applications of BNNTs are their use as nanoscale semiconductors operating in oxidative environments at high temperatures10 and as insulating nanocomposites of high thermal conductivity.10,11

Most investigations in the literature were focused on BNNTs in the solid state because these nanotubes, like their carbon counterparts, are generally insoluble in common organic and aqueous media.11 For carbon nanotubes, the research efforts on their functionalization and solubilization have stimulated and enabled the exploitation of the properties and applications that are not accessible in the solid state, such as the dispersion of carbon nanotubes in polymeric nanocomposites and the compatibilization with biological systems.12,13 Similarly significant effects on the research of BNNTs may be expected from the introduction of the nanotubes into homogeneous solutions. We report here a successful attempt in the solubilization of BNNTs.14 The solubilization was based on interactions of amino groups in oligomeric diamine-terminated poly(ethyleneoxy)poly(ethyleneoxy) (PEG) with the BNNT surface, conceptually similar to those proposed in the solubilization of carbon nanotubes.15,16 The solubilization procedure and results from the characterization of BNNTs before and after the solubilization are presented and discussed.

BNNTs were synthesized by the carbon nanotube substitution reaction,17-19 in which purified multi-wall carbon nanotubes (MWCNTs), Nanostructure & Amorphous Materials, Inc. were used as a template to react with boron oxide (powder, 99.8%, Alfa Aesar) and anhydrous ammonia (National Welders Supply Company) in a quartz chamber at high temperature (200 °C), followed by thermal treatment (780 °C) in low-pressure air to remove the residual carbon from the sample. The X-ray photoelectron spectroscopy (XPS, Kratos AXIS 165) analysis of the sample yielded a BN ratio of 1.05/1, with the slight excess of boron due to residual boron oxide. Upon further purification by repeated washing with hot water to remove boron oxide, the content of BNNTs in the sample was at least 72% by weight, comparable with those reported in the literature.10-20

The thermogravimetric analysis (TGA, Mettler-Toledo TGA/SDTA851e) result of the sample in air is shown in Fig. 1. The weight increase at high temperatures was due to the oxidation of boron in the BNNTs, and the amount of increase (−23%) was consistent with the estimated BNNT content in the sample.

The presence of BNNTs in the sample was confirmed by scanning (SEM, Hitachi S-4700) and transmission electron microscopy (TEM, Hitachi HD-2000) characterization. The SEM image in Fig. 1 (top) shows abundant nanotubes. The TEM results suggest that the BNNTs are mostly long tubes of around 20 nm in diameter (Fig. 2, bottom), with generally thinner walls than those in the starting MWCNTs and also with a distorted layer structure in the wall.

The BNNTs were solubilized via functionalization with amino-terminated oligomeric poly(ethyleneoxy)poly(ethyleneoxy) (PEG-OH, Scheme 1). In a typical experiment, a sample of BNNTs (42 mg) was mixed with PEG-OH (1000 g), and the mixture was heated at 100 °C and kept at this temperature for 3 days under nitrogen protection. Upon cooling to ambient temperature, the reaction mixture was extracted repeatedly with deionized water, coupled with centrifuging at 3000 g. According to the weight of the solid residue, the majority (more than 55%) of the starting BNNTs were solubilized in water as a result of the functionalization with PEG-OH, (Scheme 1).

The functionalized BNNTs are soluble in water and organic media compatible with PEG-OH. While BNNTs are colorless, the attachment of PEG-OH in the functionalized BNNTs changed the sample color to brown (Fig. 3). Both the functionalization and

![Figure 1](image-url)
Fig. 3 (a) An aqueous solution of the PEG_{10000}-functionalized BNNTs. (b) The suspension from the dialysis of the solution in (a) against water. (c) The supernatant from the low-speed centrifuging of the suspension in (b).

The functionalization of BNNTs with PEG_{10000} was reversible. The brown-colored solution of PEG_{10000}-functionalized BNNTs was placed in a membrane tubing (molecular weight cutoff ~12000) for dialysis against fresh deionized water. In less than 3 days, the color disappeared and the solution turned into a suspension (Fig. 3), from which colorless BNNTs were recovered almost quantitatively via simple low-speed centrifuging. As shown in Fig. 4, the FT-IR (Thermo-Nicolet Nexus 670) spectral features of the recovered BNNTs are generally the same as those of the starting BNNTs (except for several small peaks due to residual PEG_{10000} with the characteristic peaks at 1390 cm^{-1} and 800 cm^{-1} due to B-N vibrations parallel and perpendicular to the nanotube axis, respectively). Additional experiments were performed to confirm that the solution color was indeed associated with the PEG_{10000}-BNNT interactions. One way to precipitate the PEG_{10000}-functionalized BNNTs from the brown-colored solution via high-speed centrifuging. At 25000 g for 30 min, brownish precipitates were observed, and the solution became only slightly yellowish, suggesting that the color was attached to the nanotubes. In

![Scheme 1](image)

the solution color are likely due to interactions of the PEG_{10000} amino groups with nanotubes, as proposed in Scheme 1. According to results from control experiments, the amino moiety in the functionalization agent is necessary for the functionalization and solubilization of BNNTs. In fact, there was no solubilization at all when the nanotube sample was simply heated in neat deionized water under the same experimental conditions. Similarly, no meaningful solubilization was found with the use of alcohol-terminatated and methoxy-terminated PEG (PEG_{10000} and PEG_{10000}-OH, respectively) as the functionalization agent.

![Fig. 4](image)

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Fig. 5 TEM (left) and AFM (right) images of the PEG₂₀₀₀-func-
tionalized BNNTs.

another experiment, the PEG₂₀₀₀-functionalized BNNTs were
dialyzed against an aqueous solution of the PEG₂₀₀₀ with
matching concentration. No meaningful precipitation or change in
solution color were observed after the dialysis. Thus, the color
must be a part of the functionalization, as proposed in Scheme 1.

The dispersion of the PEG₂₀₀₀-functionalized BNNTs was
examined by both TEM and AFM (Molecular Imaging PicoPlus).
For the TEM specimen, a small drop of an aqueous solution of the
functionalized BNNTs was placed on a holey-carbon-coated
copper grid, followed by solvent evaporation. As shown in the
TEM image in Fig. 5, the nanotubes are well-dispersed.
The sample for AFM measurement was deposited on a mica substrate.
However, the presence of abundant functional group PEG₂₀₀₀
made the imaging somewhat difficult. Thus, the specimen was
treated at 400 °C in air for 1 h to remove some of the attached
PEG₂₀₀₀ molecules from the sample. The subsequent AFM
image of the thermally treated specimen exhibits island-like
structures on the nanotube surface (Fig. 5), which may be
attributed to the remaining PEG₂₀₀₀ functional groups.

In summary, BNNTs could be introduced into homogeneous
aqueous and organic solutions via the functionalization with
oligomeric PEG molecules bearing amino moieties. The color
change of the sample from colorless to brown upon the
functionalization was identified as being associated with interac-
tions between the PEG amino groups and the nanotube surface.
Both the solubilization and the color change were found to be
reversible, which could be interpreted mechanistically as being due
to the formation and dissociation of amine-boronic acid ionic
bonds. The solubilization via functionalization may add a new dimen-
sion in applications of BNNTs, such as their homogeneous dispersion
in nanocomposite materials for unique thermal and optical
properties.

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NMR Detection of Single-Walled Carbon Nanotubes in Solution

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Abstract: The detection of nanotube carbons in solution by 13C NMR is reported. The highly soluble sample was from the functionalization of 13C-enriched single-walled carbon nanotubes (SWNTs) with diamine-terminated oligomeric polyethylene glycol (PEG1000). The ferromagnetic impurities due to the residual metal catalysts were removed from the sample via repeated magnetic separation. The nanotube carbon signals are broad but partially resolved into two overlapping peaks, which are tentatively assigned to nanotube carbons on semiconducting (SWF) and metallic (downF) SWNTs. The solid-state NMR signals of the same sample are similarly resolved. Mechanistic and practical implications of the results are discussed.

Introduction

There have been extensive recent investigations on the functionalization of single-walled carbon nanotubes (SWNTs). The functionalization typically renders solubility of the nanotubes, enabling their characterization in homogeneous organic and aqueous solutions. NMR is one of the most desirable instrumental methods for studying the structure and properties of functionalized carbon nanotubes. However, a number of technical difficulties have probably hindered a direct 13C NMR probing of the functionalized nanotube itself in solution, such as limited sample solubility and the presence of ferromagnetic impurities, among others. Thus, the available solution-phase NMR results are centered on the characterization of the functional groups. For example, Chon et al. reported the use of 1H NMR results to validate their proposed noncovalent π-stacking mechanism for the functionalization of SWNTs with poly(ethylene ether) polymers. Holzinger et al. used 1H NMR to characterize their soluble SWNT samples functionalized by various substituted oxycarbonyl nitrene compounds. The NMR signals from the functional groups on SWNTs are often broader than those from the free functionalization agent, with generally similar patterns but sometimes shifting to the upfield.

Solid-state 13C NMR has been applied to the characterization of nanotubes in the functionalized SWNT samples. The signals of the nanotube sp2 carbons are generally broad, centered around 120–120 ppm, similar to those for unfunctionalized SWNTs. In a recent study of polymer-functionalized SWNTs, solid-state 2D 1H–13C heteronuclear correlation spectroscopy was employed for evidence of significant interactions of the functional groups with the nanotube. Even for solid-state NMR, however, it is widely acknowledged that NMR measurements and results can be negatively affected by the presence of substantial ferromagnetic impurities from the residual metal catalysts used in the nanotube production. Despite the development of various purification methods, the catalyst residues can often survive some of the rather harsh chemical and thermal treatments of carbon nanotube samples. As a result, SWNTs produced by using catalysis of nonferromagnetic metals (Ru/Pd or PtRh, for example) have been used in some recent NMR studies.

Here, we report results from the first attempt of a solution-phase 13C NMR study of nanotube carbons in functionalized SWNTs. The nanotube sample was produced with 13C isotope enrichment. The high nanotube equivalent solubility was...
achieved via the known functionalization of SWNTs with diamineterminated dendrimer poly(ethylene glycol). The ferromagnetic impurities due to the residual metal catalysts were effectively removed from the functionalized nanotube sample in solution via repeated magnetic separation. The solution-phase NMR results are compared with those from solid-state NMR measurements. The partial resolution of the carbon signals in the NMR spectra are discussed in terms of theoretically predicted differences in chemical shifts between semiconducting and metallic SWNTs.

Experimental Section

Materials. Anomalous 13C powder (99.9% carbon, 13C content >98%) and graphite powder (CVP grade) were supplied by Iota Isotopes and Bex Carbon, respectively. Carbon cement was obtained from Dyson. Powders of 13C (6.0–6.4 µm, 99.99%) and Cs (6.0–6.4 µm, 99.9%) were supplied from Alfa Aesar. 13C-D3-acetamide(13) poly(ethylene glycol) of average molecular weight, Mw ~ 1500 (PEG1500), and KCl (>99%) were obtained from Aldrich and destained silences from Cambridge Isotope Laboratories. Cellulose ester dialysis tubing with a molecular weight cut off (MWCO) of 12 000 was supplied by Sigma.

The SWNT sample without 13C enrichment was purchased from Carbon Solutions. For the purification, raw material (1.2 g) was heated in air at 300 °C for 30 min, followed by refluxing in HNO3 (2.6 M, 500 mL) for 24 h. The mixture was then cooled to room temperature and subject to centrifugation (~14 000g, Fisher Scientific Centrifuge 22C Centrifuge). The sestem was repeatedly washed with deionized water and dried under vacuum to yield a purified SWNT sample (336 mg).

Measurements. Raman spectra were obtained on a Renishaw Raman spectrometer equipped with a 50 mW diode laser source for 785 nm excitation and a CCD detector. Thermogravimetric analysis (TGA) experiments were carried out on a Mettler Toledo TGA/SDTA851e system with a typical heating rate of 10 °C/min. Electron microscopy imaging was conducted on a Hitachi HD-2000 scanning transmission electron microscope (STEM) operated at 200 kV with digital imaging capability. The atomic absorption analysis service was provided by Goldie and Associates (Secaucus, New Jersey). Samples for the analysis were digested by using hot HNO3/HCl-mixed acid in accordance with the EPA 2002 method.

NMR measurements were performed on a Bruker Avance 500 NMR spectrometer equipped with a 4 mm magic angle spinning (MAS) probehead for solids and a 5 mm micro-probehead for solutions. For very broad signals, exponential multiplications with a line broadening up to 500 Hz was applied for each carbon FID (free induction decay), coupled with user-defined spline baseline correction in the data processing. The spin–lattice relaxation times of both solid and solution samples were measured with the inversion recovery pulse sequence. Since the solution and solid-state NMR experiments showed that the nanotube carbon signals were not affected by the proton decoupling, the reported NMR spectra were collected without the decoupling (to avoid overheating the sample and potential damage to the equipment in solid-state experiments).

13C-Enriched SWNTs. The laser ablation method(13) was used for the synthesis of 13C-enriched SWNTs. The laser source was a Spectra Physics Quanta-Ray PRO-280 Q-switched Nd:YAG laser operated at 16 Hz (2 Pyruvate at 1044.4 cm and 9 mm beam diameter). In a typical experiment, the ablation target was prepared by mixing powdered 13C (0.40 g), graphite (1.25 g), Ni (0.236 g), and Co (0.236 g) with graphite cement (29 g) for hotpressing (110 °C) into a pellet (about 10 mm thick and 18 mm in diameter), followed by baking at 180 °C for 5 h in air, curing at 310 °C for 3 h, and annealing at 1200 °C for 5 h in argon flow (50 scmn, atmospheric pressure). The furnace temperature was set at 1150 °C with a steady argon flow (52 scmn, 75 Lpm), in the ablation experiment. The rubber-like carbon sol from the laser ablation was characterized by Raman, and the results were consistent with the expected substantial presence of 13C-enriched SWNTs in the sol.

According to the characteristic G-band shift, the atomic content of 13C is the nanotube was estimated as 10%.

The purification of the 13C-enriched SWNT sample was similar to that discussed above for the regular SWNT sample.

Functionalization and Magnetic Separation. In a typical experiment, a purified 13C-enriched SWNT sample (60 mg) was mixed with Fe3O4@OD (1.2 g), and the mixture was heated to 125 °C for 3 days at that temperature, the reaction mixture was cooled to ambient for repeated extraction with water. In each extraction, the solution filtration containing the Fe3O4@OD-functionalized nanotubes was separated from the insoluble residue via centrifuging at ~4000 g for 15 min. Typically three repeats were performed, with the supernatant in the last repeat being colorless. The aqueous solutions from the repeated extractions were combined for magnetic separation.

The magnetic separation to remove residual metal catalysts in the solubilized sample was accomplished by using a commercially available magnetic separator (Dynam Biotech Model MPC-4.1). Each separation experiment was for 2 days, and the experiment was repeated three times to ensure maximal precipitation of all magnetically responsive species. The final supernatant was recovered, followed by digestion (MWC0 ~ 12 000) against fresh deionized water for 3 days (removing free Fe3O4@OD) to yield a colorless aqueous solution of Fe3O4@OD-functionalized 13C-enriched SWNTs (PEG1500–13C–SWNT).

The same procedure was applied to obtain a magnetically purified Fe3O4@OD-functionalized SWNT sample without 13C enrichment for the nanotubes.

Results and Discussion

The properties (appearance, solubility, nanotube content, etc.) of the PEG1500–13C–SWNT sample are similar to those of their counterpart without 13C enrichment already reported in the literature.(15) The high solubility of these functionalized nanotubes, coupled with the 13C enrichment, make it possible to probe nanotube carbons in solution-phase NMR measurements. As shown in Figure 1 for PEG1500–13C–SWNT in D2O (solution concentration ~ 36 mg/mL, SWNT equivalent), the nanotube sp2 carbons exhibit a broad signal centered at ~132 ppm (fwhm = ~2.8 ppm), which is consistent with theoretical predictions(16) and close to those observed in solid-state NMR.6 Obviously, nanotube carbons can be detected by NMR in solution.

The broadness in the signals reflects the chemical shift dispersion of nanotube carbons, which are likely inhomogeneous due to different nanotube chiralities, lengths, adjacent defects, etc.(16) Interestingly, however, there are some distinctive features in the broad signals, which through deconvolution (resolving the curve into underlying peaks) can be represented by two Lorentzian peaks of similar line-widths (~20 ppm, Figure 1). The ratio of area under the peak centered at 128 ppm to that at 144 ppm is ~1.8. A variation of relaxation delay time from 0 to 4 s had little effect on the signal shape, with similar line-widths and chemical shifts. We tentatively assign the two peaks to the VC(11)


Figure 1. The $^{13}$C NMR spectrum of PEG$_{2000}$--$^{13}$C-SWNT in D$_2$O solution (3000 45° scans, 2 s relaxation delay, acquired in CP-MAS probe without spinning and decoupling). Shown in the inset is a deconvolution based on two Lorentzian peaks (reproduced curve, ---). The 78 ppm signal is due to nanotube-attached PEG functional groups.

to semiconducting (upfield) and metallic (downfield) SWNTs. In fact, the observed difference in their chemical shifts (~16 ppm) is in reasonable agreement with what has been predicted by recent theoretical calculations. These calculations suggested that there should be an approximately ~12 ppm upfield shift for the semiconducting nanotube carbons from their metallic counterparts due to the localized ring currents.

There was also a suggestion that the broad solid-state NMR signals of nanotube carbons could be deconvoluted into two peaks corresponding to semiconducting and metallic SWNTs, despite the fact that those signals were not resolved at all.

The peak shoulder structure shown in Figure 1 represents the first experimental confirmation that there is indeed a pair of broad signals associated with the sp$^2$ carbons in SWNTs. The acquisition of the partially resolved $^{13}$C NMR benefited significantly from the effective removal of residual metal catalysts via repeated magnetic precipitation in solution. It is well-known that ferromagnetic residues associated with the Ni-Co catalysts interfere with NMR measurements. These residues are often encapsulated in carbon cages or structural cavities and are, thus, impossible to remove completely in the chemical purification. The solidification of the nanotube sample enabled the solution-phase magnetic separation. The separation was effective, as confirmed by STEM analyses of the separated samples (Figure 2) and by TGA analysis (negligible residue), and also was reflected in the NMR results (diminished spinning sidebands in the solid-state NMR spectrum, for example). According to the atomic absorption analysis of the PEG$_{2000}$--$^{13}$C--SWNT sample, the Ni content was <0.067 wt % and the Co content was much lower (below the detection limit).

The solid-state $^{13}$C MAS NMR spectrum of PEG$_{2000}$--$^{13}$C--SWNT (in a mixture with KCl, 10 000 90° scans, 2 s relaxation delay, 14 kHz spinning rate, single pulse sequence, no decoupling) of the PEG$_{2000}$--$^{13}$C--SWNT sample was acquired for comparison with the solution-phase result. The nanotube carbon signals in the solid-state spectrum are equally broad, with two obvious overlapping peaks at ~128 and ~136 ppm (Figure 3). Similar to the solution-phase signals, these two peaks may also be assigned to semiconducting and metallic SWNTs. The relative intensities

Figure 2. STEM images of the PEG$_{2000}$--$^{13}$C--SWNT sample (in secondary electron mode, top) and the precipitate from magnetic separation (in L-contrast mode, bottom). The L-contrast imaging of the PEG$_{2000}$--$^{13}$C--SWNT sample revealed no metal. Scale bars = 100 nm.

Figure 3. The solid-state MAS $^{13}$C NMR spectrum of PEG$_{2000}$--$^{13}$C--SWNT (in a mixture with KCl, 10 000 90° scans, 2 s relaxation decay, ~14 kHz spinning rate, single pulse sequence, no decoupling).

(18) The functionalization may be attributed to isocyanate between the amino groups on PEG$_{2000}$ and nanotube-bound carbonylic acids.

(19) The absorption of amino moieties into the nanotube surface is also difficult to expect that there could be enough difference in the functional group coverage on the nanotube surface to cause bands in the poorly resolved peaks in nanotube carbon signals. Nevertheless, it remains a possible alternative in the explanation of these signals.


of the two peaks are somewhat different in solid state versus in solution. Additionally, the overall intensity of the nanotube carbon signals in reference to that of PEG$_{1000}$ functional groups is significantly higher in solid state than in solution. These two differences between solid-state and solution-phase NMR results may share the same cause. As in other soluble functionalized SWNTs, the PEG$_{1000}$-$^{13}$C$-$SWNT sample contains bundled nanotubes in solution. The tumbling of larger bundles may proceed too slow to eliminate such orientation-dependent contributions to the NMR line-width as chemical shift anisotropy and dipolar coupling. These species are essentially NMR “silent” in solution, corresponding to a lower effective nanotube carbon concentration to result in their relatively weaker overall signal intensity in the solution-phase NMR spectrum. The functionalized semiconducting SWNTs disperse better in solution, as made evident by recent experimental results. Therefore, their NMR signals relative to those of their metallic counterparts are stronger in solution (Figure 1) than in the solid state (Figure 3).

There are apparently significant interactions between the nanotube carbons and the PEG moieties in the solid state, with the latter serving as spin–lattice relaxation centers. The relaxation time ($T_1$) of the nanotube carbons was estimated by using the null-point approach based on the Inversion Recovery sequence. Both nanotube components effectively “disappeared” at the same point, $t_{null} \sim 0.16$ s, corresponding to $T_1 \sim 0.2$ s. Despite the absence of ferromagnetic impurities in the PEG$_{1000}$-$^{13}$C$-$SWNT sample, the estimated $T_1$ is up to 2 orders of magnitude shorter than those of similarly $^{13}$C-enriched SWNTs without functionalization. The interactions are also reflected in the NMR spectra of the PEG carbons. For PEG$_{1000}$-$^{13}$C$-$SWNT in D$_2$O solution, the spin–lattice relaxation time of PEG carbon signals (550 ms) is close to that in free PEG$_{1000}$ (710 ms). However, in solid state, the relaxation time of PEG carbons in the PEG$_{1000}$-$^{13}$C$-$SWNT sample is more than an order of magnitude shorter (37 ms) than that in free PEG$_{1000}$ (430 ms). It seems that the segmental mobility of PEG moieties in PEG$_{1000}$-$^{13}$C$-$SWNT in the solid state is low, presumably with the motion of PEG carbons restricted by their proximity to the nanotubes. Such significant difference of the relaxation times for the nanotube-bound functional groups in solution phase versus in solid state is interesting. It may be exploited for potential applications in the NMR characterization of nanocomposite materials, such as probing interactions of carbon nanotubes with the polymeric matrix.

In summary, we demonstrated that the nanotube carbons in solution could readily be detected in $^{13}$C NMR by using highly soluble functionalized SWNTs. The ferromagnetic impurities in the sample for NMR measurements were effectively removed via repeated magnetic separation. The nanotube carbon signals are broad, but partially resolved into two adjacent features, probably corresponding to nanotube carbons in semiconducting (upfield) and metallic (downfield) SWNTs. The solid-state NMR signals of the same sample are similarly resolved. These results suggest that $^{13}$C NMR may be exploited to serve as a useful tool in the characterization of SWNTs of different electronic structures.

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Supporting Information Available: Raman spectra of $^{13}$C-enriched SWNTs and SWNTs without enrichment. This material is available free of charge via the Internet at http://pubs.acs.org.
