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Preparation and Studies of Carbon Dots

Fan Yang
Clemson University, yfbobo0218@gmail.com

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PREPARATION AND STUDIES OF CARBON DOTS

A Dissertation
Presented to
the Graduate School of
Clemson University

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

by
Fan Yang
May 2018

Accepted by:
Dr. Ya-Ping Sun, Committee Chair
Dr. Brain Dominy
Dr. Andrew G. Tennyson
Dr. Daniel C. Whitehead
ABSTRACT

Carbon dots, a new class of benign fluorescent nanomaterials, have been widely studied over the past ten years, as reflected by the recent exponential growth of publications. Such properties as their optical performance, physicochemical and photochemical stability, and aqueous solubility enable them to serve as bright optical probes in a variety of imaging and sensing applications. This dissertation explores the essence of carbon dots, systemically comparing them to graphene quantum dots, thus providing a reexamination of the former. It provides an enhanced understanding of carbon dots from a structural and mechanistic perspective based on the results from their functionalization and defunctionalization. This study also analyzes the advantages of the optical and redox properties of carbon nanoparticles in comparison to other zero-dimensional carbon allotropes, specifically fullerenes, functionalized by electronic polymers. The results should prove interesting and valuable for the further application of carbon dots in various optoelectronic devices and systems.
DEDICATION

To

my father Mr. Keyi Yang and my mother Mrs. Xiumei Zhang
I would like to thank my advisor, Dr. Ya-Ping Sun, for his full support, enlightening advice and great patience throughout my Ph.D. career at Clemson. His unremitting passion on research serious attitude towards life have deeply impacted my views and urged me to mature. It was his guidance helped me to go through all those hard times during my research process and build up a solid foundation for my future life.

I would also like to express my appreciation to my Ph.D. committee members, Dr. Brain Dominy, Dr. Andrew Tennyson and Dr. Daniel Whitehead, for their timely help and kindly suggestions during my pursue of Ph.D.

To the past and present members of Dr. Sun’s lab, I sincerely want to express my gratitude for all the support and help. To the past members, Dr. Mohammed Meziani, Dr. Sumit Sonkar, Dr. George Luo, Dr. Yin Hu, Qingwu Xiong, and Jiayu Song and the current members, Yamin Liu, Gregory LeCroy, Weixiong Liang and Dr. Xianyan Ren, I would like to thank you all for being the absolutely necessary roles during the most valuable time of my life so far. I would emphasis my thanks to Mrs. Ping Wang for her help and advice whenever I asked for it.

To all my dear friends in Clemson, I would cherish all those memories we shared together. Zhengxin Wang, Lynn Zhang, Zhe Jia, Jerry Jiang and Yi Jin, it was your consistent encouragement and support stayed with me all the way to this point of time. Our friendship is going to warm my heart for a long time and I would definitely be proud of this journey because of you all whenever I recall it later.
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CHAPTER ONE
CARBON “QUANTUM” DOTS AND GRAPHENE QUANTUM DOTS: SYNTHESSES,
PROPERTIES, AND APPLICATIONS

1.1 Introduction

Recently, fluorescent materials have been the focus of much research attention due to their unique chemical and physical properties and, therefore, multiple applications, including sensing, imaging, targeting, and photovoltaic devices, among others. The most significant uses, semiconductor nanocrystals of physical dimensions smaller than the exciton Bohr radius commonly referred to as quantum dots (QDs), have seen wide-spread application since their development in the early 1970s. QDs are known for their advantages over the more conventional fluorescent materials such as organic dyes and polymer conjugates. Because of such properties as strong optical absorption, high fluorescence emission quantum yields, large Stokes shifts, and excellent resistance to photobleaching, they have been introduced into many fields, with their importance being recognized by the 2000 Nobel Prize in Physics awarded to Alferov and Kroemer. With their commercial availability, it is believed that QDs will be one of the most well-known fluorescent materials.

However, the primary disadvantage of QDs is their toxicity due to heavy metals such as cadmium and lead which are commonly found in them. This toxicity has significantly limited their application in most bio-related fields, which may ultimately keep them from the desired fluorescent materials. As a result, benign nanomaterials with...
comparable or even better performance than QDs are in demand. Recently, several promising candidates have been developed, among which carbon-based fluorescent materials, which are the most exciting, have seen rapid development.$^{20-27}$
Figure 1.1 Cartoon illustration of carbon dots (top) and GQDs (bottom). (From Ref. [27] and [32])
Carbon “quantum” dots (or more appropriately called carbon dots for their lack of classical quantum confinement behavior) and graphene quantum dots (GQDs) have emerged as the two leading classes of carbon-based fluorescent materials, with others such as graphene oxide quantum dots and carbon nanotube quantum dots having potential but requiring more research. Carbon dots, initially reported by Sun et al. in 2006,28 are defined as small carbon nanoparticles with various particle surface passivation schemes (Figure 1.1). They are usually synthesized through the introduction of defects onto the surface of the carbon core via various functionalization processes as reported in several publications28-36 coming from such sources as laser ablation of graphite,28 carbonization of plants,32 pyrolysis of small molecules,33 and at times unusual materials.34-36 While the surface functionalization groups can come from different polymers or monomers,30-32 which containing amino groups are preferred as they enhance the optical property of carbon dots compared to other option such as hydroxyl groups.

GQDs, first synthesized by Eda et al. in 2010,37 are theoretically small fragments of single-layer graphene sheets or the equivalent configuration of conjugated π-islands in a single-layer graphene sheet, thus without an interlayer quenching effect, which could be substantial (Figure 1.1). The creation of conjugated π-domains is the common strategy used in GQDs synthesis, with typical methods including cutting graphene sheet into nanofragments and growing nanoscale graphene via chemical vapor deposition (CVD);38 however, most reported GQDs consist of layered nanoscale-reduced graphene oxides (rGOs) rather than the strictly defined configuration, resulting in more defect-derived contributions to the optical properties than those observed in the single-layer
configuration.\textsuperscript{37-40} GQDs as well as carbon dots have exhibited such advantages as aqueous solubility, high optical performance, and nontoxicity, making them viable for bioimaging and photocatalytic energy conversion.\textsuperscript{21,23,25}

Although it may appear quite different from their definitions, carbon dots and GQDs are related based on a careful review of the most significant publications. Because of the limitations of the characterization methods and this less than obvious connection between the two, it is sometimes difficult to differentiate one from the other; thus, it is not surprising that at times what articles refer to as carbon dots are more accurately GQDs or vice versa. This chapter focuses on the differences and similarities between carbon dots and GQDs, then showing how these differences converge to a certain degree, creating a connection that links them. Unlike previous reviews of the literature comparing carbon dots and GQDs, this chapter integrates this past research in more detail and more directly through a new lens focused on their synthesis and properties.

1.2 Syntheses

Many methods have been developed for fabricating GQDs and carbon dots over the past ten years. Based on the resulting dot structure, which is associated with their optical properties, these methods can be classified as the creation of conjugated $\pi$-domains and the introduction of defects via functionalization, these two categories corresponding to the current strategies for the preparation of GQDs and carbon dots, respectively, for developing various synthetic routes. While in some cases the two may be applied jointly as the creation of conjugated $\pi$-domains is often achieved by
manipulating defects in the graphene sheets, thus resulting in the synthesis of functionalized GQDs, which will be discussed later.

1.2.1 Creation of Conjugated π-Domains

GQDs have primarily been synthesized through the creation of conjugated π-domains. Past research has found that a single layer of graphene sheet should be non-fluorescent due to the lack of electronic bandgaps. However, when isolating sp² islands (such as graphene fragments with a finite Bohr radius), the quantum confinement effect becomes significant. The typical routes are usually created by “cutting” graphite into small fragments using acidic oxidation and hydrothermal treatments, a procedure Eda et al. reported as resulting in the bandgap opening in the reduced graphene oxide (rGO). In their study, GOs prepared using a modified Hummer's oxidation method were reduced via exposure to hydrazine into rGOs, in which the graphene π network was disconnected, equivalent to the formation of isolated π-domains (approximately 3 nm in size edge-to-edge) in the carbon-oxygen sp³ matrix. These quantum-sized sp² islands have the potential to localize the electron-hole pair, thus creating energy bandgaps resulting in fluorescence emissions via radiative recombination (Figure 1.2).
Figure 1.2 Structural models of GO at different stages of reduction (upper), and the energy gap of $\pi-\pi^*$ transitions calculated based on DFT as a function of the number of fused aromatic rings (lower). (From Ref. [37])
Similarly, Pan et al. reported GQDs with strong blue fluorescent emissions were prepared from cutting oxidized graphene sheets (GSs) using a hydrothermal process, the diameters of these synthesized GQDs primarily being in the range of 5-13 nm, with an average of 9.6 nm, and more than 85% consisting of 1-3 layers of oxidized GSs. The fluorescent quantum yield measured was 6.9% when GQDs were excited at 320 nm. In addition, the fluorescence was wavelength dependent, its intensity decreasing rapidly when excited at longer wavelengths. These researchers hypothesized that this blue emission might originate from free zigzag sites with a carbene-like triplet ground state. Furthermore, they reported that the blue photoluminescence from GQDs were pH dependent as the enhanced fluorescence was observed under alkaline conditions.

Anathanarayanan et al. developed a highly efficient strategy for synthesizing GQDs from electrochemical cutting graphene with a high throughput. In this method, the precursor 3D graphene was prepared through chemical vapour deposition (CVD), followed by electrochemical exfoliation in an ionic liquid at room temperature. The ionic liquid used in this synthesis not only assists in the exfoliation and dispersion of GQDs but also gives the GQDs a sensitivity for optical detection of Fe$^{3+}$ ions. The synthesized GQDs were of high crystallinity and uniform distribution in lateral diameter ($\approx$3 nm) and thickness (primarily single-layered). The same electrochemical process when applied to 2D CVD-grown graphene, however, resulted in electrical discontinuity and a poor yield of GQDs.
The creation of conjugated π-domains is the primary strategy for the synthesis of GQDs, with the most practical method involving cutting graphene into small fragments. If it is for homemade graphene as precursor; an alternative method would involve potentially controlling the size during graphene growth, with the aim of developing the final product instead of focusing on the growth first and then cutting to fit the size requirement. Despite the rapid growth in the field of GQDs, most results are still based on theoretical calculation (such as using density functional theory) since pure GQDs have not yet been created through experimental methodologies. The reported GQDs obtained through experiments frequently contain such heteroatoms as O, S and N, which are introduced during the cutting process no matter by which means; thus, they resemble functionalized GQDs, in which surface defects play an important role in the optical properties, similar to those found in carbon dots.

1.2.2 Introduction of Defects via Functionalization

While the creation of conjugated π-domains has been widely applied in the synthesis of GQDs, the resulting dots with weak fluorescent emissions in the visible region are not quite desirable. Carbon dots have been well-known for their full-color emission and bright fluorescent performance since their development, and based on subsequent research, they have been defined as surface functionalized carbon nanoparticles. The purpose of functionalization is to introduce “defects” on the surface of carbon nanoparticles, a crucial step in response to the need for excellent optical properties. These defects, defined here as any site other than the perfect sp² domains,
usually contain carboxylic or hydroxyl groups, or amide bonds introduced via oxidation, chemical reaction or physical absorption.\textsuperscript{31,42,43}

The first synthesis performed by Sun \textit{et al.}\textsuperscript{28} functionalized carbon nanoparticles prepared from laser ablation (also known as deliberate functionalization) with diamine-terminated polyethylene glycol (\textit{PEG}_{1500N}) or poly-(propionylethylenimine-\textit{co-}
ethylenimine) (PPEI-EI). The resulting synthesized carbon dots were 5 nm in diameter, with emissions covering the visible wavelength range and extending into the near-infrared (Figure 1.3) and the observed quantum yields being from 4\% to more than 10\% at 400 nm excitation. Additional research conducted by Wang \textit{et al.} found that as-prepared PEG\textsubscript{1500N}-carbon dots could be processed by separation on an aqueous gel column,\textsuperscript{31} the resulting fraction with the most fluorescence exhibiting quantum yield close to 60\% (excitation at 440nm and emissions centered around 520 nm). Of importance is the fact that although as-prepared carbon dots are very fluorescent, the surface functionalization groups contain no visible or near-UV chromophores, meaning they are not emissive at visible wavelengths.\textsuperscript{31}
Figure 1.3 Aqueous solutions of the as-synthesized PEG$_{1500}$-carbon dots excited at the indicated wavelengths in nm and photographed directly (top), and excited at 400 nm and photographed through band-pass filters of different wavelengths as indicated (bottom). (From Ref. [28])
The continued success of applying surface functionalization in the synthesis of carbon dots exhibiting excellent properties has led to much carbon dot research focused on various novel or facile routes\textsuperscript{44-50}, though most of them are either essentially different forms of the functionalization process or having it within the process. Among those methods, the one-pot syntheses appear to be the focus of most of this attention. However these syntheses are sometimes misunderstood as being capable of producing brightly fluorescent carbon dots without surface functionalization although it appears likely that it is embedded rather than being a separate procedure.

Jiang et al. prepared silicon-carbon dots@dopamine using (3-aminopropyl) triethoxysilane, glycerol, and dopamine (DA) as raw materials via a one-pot microwave-assisted method,\textsuperscript{51} indicating that during the process of microwave-assisted irradiation, glycerol was carbonized and, thus, acted as a carbon backbone with both (3-aminopropyl) triethoxysilane and DA as the functionalization groups attaching to the surface via an amidation reaction, though detailed structural information was not provided. The synthesized dots exhibited similar optical properties as typical carbon dots with the fluorescence emission quantum yield of 12.4\% when excited at 400 nm and the size distributed in the 8-15 nm range with an approximate average of 10 nm. Wang et al. reported a similar one-step microwave-assisted approach using phosphorus-rich phytic acid (PA) as the carbon source and ethylenediamine as the surface passivation groups to prepare water-soluble, phosphorus-containing and highly green fluorescent carbon dots.\textsuperscript{52} The synthesized carbon dots were further purified through acetone extraction, with the resulting carbon dots showing a high quantum yield of 22\% when excited at 400 nm.
More recently, Jiang et al. succeeded in the preparation of three carbon dots emitting red, green and blue colors under a single ultraviolet-light excitation from the one-pot solvothermal of three isomers of phenylenediamines (p-, o-, and m-phenylenediamine), all three of which were uniform with average sizes of approximately 10.0 nm (p), 8.2 nm (o), and 6.0 nm (m) and with quantum yields of 20.6%, 10.4%, and 4.8%, respectively, when excited at 365 nm. The fluorescent lifetimes, however, were not consistent with the typical carbon dots (multi-exponential decay and relatively long lifetime), with the fluorescent decays of o-carbon dots and p-carbon dots in an ethanol solution being mono-exponential with lifetimes of 4.44 and 9.39 ns respectively, and only the fluorescent decay of the m-carbon dots being fitted with a bi-exponential function but with a much shorter average lifetime of 0.99 ns.

As this analysis suggests, the deliberate functionalization approach has been successful in producing structurally well-defined carbon dots with high fluorescence quantum yields (more than 50% in some configurations), but the synthesis is tedious and subject to limitations in the selection of molecules for functionalization. The one-pot approach is more efficient and versatile, compatible with a diverse selection of precursors and functionalization molecules or species, but less controllable both in the synthesis and for the structures of the carbon dots produced, among other processing and performance issues.

The defect-generation based method is not strictly applied to carbon dots only, as according to recent studies, GQDs can also be further functionalized purposely, with the
resulting functionalized GQDs being very close to carbon dots.\textsuperscript{55-58} Jin \textit{et al.} observed that GQDs prepared from cutting GOs into fragments upon surface functionalization with PEG-diamine (GQDs-NHR) were considerably brighter than that of GQDs without functionalization,\textsuperscript{58} and the fluorescent emission also red-shifted by 28 nm after functionalization. Furthermore, the fluorescence shift behavior of the GQDs through functionalization was confirmed using DFT calculations. Both experimental and computational analyses revealed that the charge transfer between functional groups and GQDs can tune the band gap of the GQDs and, consequently, the fluorescent emission due to the changing electron density in the latter.

While the clear physical structures of GQDs and carbon dots are still unknown to a certain degree, it is not appropriate to use the names interchangeably because of the widely accepted definitions and formation mechanisms as this lack of exactitude would confuse the public. Yu \textit{et al.} introduced an approach for preparing carbon dots by non-focusing pulsed laser irradiation in toluene,\textsuperscript{59} a formation process they hypothesized involved the growth of graphene followed by ablation into small fragments, which were suggested to be responsible for the fluorescence observed. However, it is more appropriate to define the product as GQDs rather than carbon dots as the characteristics of the ablated graphene fragments fit the definition of the former. Oza \textit{et al.} demonstrated a green synthetic route for carbon dots starting from a natural precursor, camphor.\textsuperscript{60} However, their step-wised transformation of camphor to carbon dots began with the growth of graphite from camphor molecules, followed by oxidative exfoliation to GO,
and then reduced with NaBH₄ to yield carbon dots. This step-wised synthesis is essentially the classic route for GQDs, namely cutting graphite into small fragments.

These two strategies are classified based on their products—typically defined as GQDs and carbon dots, definitions which focus on the intrinsic characteristics of the dots rather than the method itself. In other words, there are several ways to cut graphite into GQDs, including oxidation/exfoliation, an electrochemical process, and hydrothermal treatment, among others and there is no mention of a functionalization process in carbon dots synthesis, but the core idea is the same despite various tools. As mentioned previously, the two strategies are not isolated, and actually they co-function in many cases.

1.3 Properties of GQDs and Carbon Dots

1.3.1 Optical Properties

1.3.1.1 Absorption

GQDs generally show strong optical absorption in the UV region but weak in the visible (Figure 1.4a). For conventional QDs, the quantum confinement effect has been repeatedly verified experimentally. Based on the same bandgap theory, GQDs should exhibit similar size-dependent effects; however, it is a challenge to prepare GQDs with a narrow size distribution for study. To address this issue, Tang et al. developed a novel method for preparing water-soluble monodispersed GQDs with diameters ranging from 1.5 to 3.9 nm, integrating soft template, microwave, and hydrothermal techniques into
one simple approach. The absorption spectra observed from the as-prepared GQDs of various diameters demonstrated a similar size-dependent effect, though not as significant as conventional QDs. It was found that both absorption peaks of 228 and 282 nm became stronger as the diameter increased, while the intensity of the absorbance increased exponentially with their increasing diameters. The absorption peak at approximately 228 nm was attributed to the $\pi-\pi^*$ transition of C=C bonds and the peak at approximately 282 nm to the n-$\pi^*$ transition of C=O bonds, which is introduced during the hydrothermal process.\textsuperscript{55} Further size-dependent studies using more controllable and scalable techniques are needed.
Figure 1.4  a) UV/Vis absorption (solid line) spectrum of GQDs in aqueous solution prepared from citric acid and ethylenediamine. Insets show photographs of GQDs solution under visible (left) and UV (right) light. b) Absorption spectrum of the EDA-carbon dots in aqueous solution (photograph in the set). c) Fluorescence spectra of GQDs in aqueous solution corresponding to a) excited at the indicated wavelengths. d) Fluorescence spectra of EDA-carbon dots in aqueous solution excited at the indicated wavelengths. (From Ref. [26] and [33])
On the other hand, carbon dots are generally more efficient in absorption of long wavelengths in the visible region due to the \( \pi \)-plasmon electrons of the core carbon nanoparticles (Figure 1.4b), with an estimated molar absorptivity of 50–100 \( \text{M}_\text{C-atom}^{-1} \text{cm}^{-1} \) in the 400–450 nm region, where \( \text{M}_\text{C-atom} \) denotes the molar concentration in terms of carbon atoms in the core carbon nanoparticles of the carbon dots in the aqueous suspension. For example, LeCroy et al. synthesized carbon dots with 2,2’-(ethylenedioxy)bis(ethylamine) (EDA) molecules as the surface passivation agents,\(^{30}\) with the as-prepared EDA-carbon dots representing less than 5 nm of the overall dot diameter. As shown in Figure 1.4b, the absorption spectra observed indicated an absorptivity for EDA-carbon dots with a carbon core of 3.5 nm in diameter of up to approximately 250 000 \( \text{M}_\text{C-particle}^{-1} \text{cm}^{-1} \), where \( \text{M}_\text{C-particle} \) refers to the molar concentration of the carbon dots with 3.5 nm diameter carbon cores.

1.3.1.2 Fluorescence

Fluorescence is probably the most appealing optical property of carbon-based quantum dots. GQDs usually exhibit a blue emission with a maximum intensity excited below 370 nm, and the quantum yields are generally low, especially in the visible region, similar to those found in “naked” carbon nanoparticles.\(^{64-70}\) In addition, wavelength independent fluorescence is often observed with regard to GQDs, with an emission peak usually at 420–450 nm (Figure 1.4c). In contrast, carbon dots exhibit full color emission all the way to near-IR, with the maximum emissive intensity dependent on the synthetic
The fluorescent quantum yields of carbon dots are much higher than those of GQDs, which are comparable to those of well-established QDs.

Wavelength dependent fluorescence is another characteristic property of carbon dots, with the typical fluorescence spectra observed progressively red-shifting in response to longer excitation wavelengths (Figure 1.4d). This wavelength dependence of fluorescence is probably related to the inhomogeneity of the carbon dots, a result of uncontrollable synthetic routes. Unlike the conventional QDs, for which the fluorescence observed is largely dependent on the size (known as “quantum confinement”), it is much harder to prepare carbon dots of uniform size; even though a few reports of some success have been published, the surface functionalization typically occurs randomly, resulting in many emissive sites accounting for the dependence on the wavelength observed in the fluorescence. Thus, the tunable fluorescent carbon dots reported in some research should be differentiated from the conventional QDs, as their tunability is probably not based on quantum confinement as it is in QDs, but resulting from the inhomogeneity induced wavelength dependent property, which allows for carbon dots with multi-colors at different excitation wavelengths, a characteristic important in certain practical applications.

The bright emission in the visible region and the wavelength dependent property are almost always associated with surface functionalization; thus, well-defined carbon dots include both, and similar characteristics have also been observed in the well-functionalized GQDs. Qu et al. prepared S, N co-functionalized GQDs (SNGQDs) from
citric acid and thiourea via a hydrothermal process. These as-prepared SNGQDs exhibited excitation independent fluorescence when excited in the region of 300-380 nm, but became excitation dependent when excited in the visible region. In addition, based on their fluorescence decay study, the fluorescence lifetime of SNGQDs was 12.8 ns under the excitation of 360 nm, a much longer period of time than that of GQDs with little surface functionalization. Much research has demonstrated carbon dots exhibiting a similar wavelength independent property in the UV region as that of GQDs, though it is not as interesting as in visible region. For example, Chen et al. reported carbon dots prepared from a one-pot hydrothermal treatment of poly(vinyl alcohol) (PVA) and ethylenediamine, in which PVA served as the carbon resource and ethylenediamine as the surface passivation agent. The synthesized carbon dots exhibited wavelength independent fluorescence emissions when excited below 340 nm with the fitted emission peak at 414 nm, while the dots became wavelength dependent when excited from the 340 nm into the visible region.

Based on optical properties (primarily absorption and fluorescence), the strength of GQDs lies in the UV rather the visible region, while carbon dots exhibit an overall strength in relation to their UV-related properties. These characteristics depend on both dots’ structures and consequently impact their further application.

1.3.2 Fluorescence Origins

Despite recent wide-spread interest in carbon-based quantum dots, mechanistic studies remain limited. One important research focus centers on the issue of the origin of
their fluorescence, with many studies attempting to interpret this unique phenomenon. However, a traditional theory such as molecular orbital is not able to fully explain the various features of this new fluorescent material since carbon dots and GQDs are more complicated than molecules, and computational simulation, which can provide only an approximate idea, is problematic when applied to such a complex system. This chapter selects two of the most widely accepted current explanations of the origin of the fluorescence, bandgap transition and defect-derived fluorescence, both of which correspond to the two major synthetic strategies discussed above.

1.3.2.1 Bandgap Fluorescence of Conjugated $\pi$-Domains

Pristine graphene, which has an extended sp$^2$ honeycomb carbon network, is a zero bandgap material, meaning in theory it emits no fluorescence. However, when its infinitely uniform structure is disrupted by, for example creating isolated sp$^2$ islands during a large graphene process, the resulting bandgaps created allow for fluorescent emissions. No connections are expected between those $\pi$-domains to address interisland quenching. To do so, it is produced as a sheet, which is then cut into fragments of conjugated $\pi$-domains or the equivalent a technique which, generally speaking, explains the phenomenon observed such as the blue fluorescence emissions which match the calculated bandgap energy and the red shifted emission as the size of the GQDs increases. Lim’s group found that the fluorescence of GQDs can be sensitively tuned by size (Figure 1.5). Using density-functional theory (DFT) and time-dependent DFT calculations, they found that the red-shift of emission wavelengths resulting from the
increasing size of GQDs results from the decrease in the bandgap caused by $\pi$-electron delocalization. According to their calculations, a pristine armchair-edged GQD consisting of 132 conjugated carbon atoms should emit at 667.4 nm, a value close to the experimental results (670 nm).
Figure 1.5 Calculated emission wavelength (nm) using the TDDFT method in a vacuum as a function of the diameter of GQDs. (From Ref. [89])
The bandgap transitions exhibit weak or no emissions in the visible region, as the fluorescence quantum yields observed are very low (less than 2%).\textsuperscript{37} This weak fluorescence is probably associated with the lack of surface passivation. The conjugated $\pi$-domains result in a strong absorption in the UV region, but the excitons are possibly largely quenched by non-radiation relaxation. In addition, the as-prepared GQDs are typically studied in dispersion, meaning that the passivation by surrounding solvent molecules should be considered as it has been reported that carbon-based dots performed differently depending on the solvent.\textsuperscript{64} Thus, it has not been reported that GQDs exhibit fluorescence emissions in solid form, while carbon dots, on the other hand, have been applied in fabricating films with strong fluorescence.\textsuperscript{90}

1.3.2.2 Fluorescence of Defect-Derived Origins

The bandgap fluorescence requires single-layer configuration of isolated sp\textsuperscript{2} islands in order to avoid interlayer quenching.\textsuperscript{91} However, the closest configuration among the reported GQDs thus far is of 1-3 layers.\textsuperscript{37,39,40} Although when single-layer fragments exist, significant interlayer quenching could drain the radiative relaxation to a great extent; thus, the fluorescence observed must originate from another source. Because the as-prepared GQDs contain hydroxyl and carboxyl groups primarily introduced during the synthetic process, which essentially creates sp\textsuperscript{3} carbon defects on the perfect sp\textsuperscript{2} domains (Figure 1.6), it is hypothesized that the fluorescence emissions arise from surface-related defective sites, generally those other than the sp\textsuperscript{2} domains.\textsuperscript{41} The
fluorescence of Carbon dots is the typical example that can be explained by defect-derived origins.
Figure 1.6 Top: (right) isolated sp2 islands in a graphene sheet and a photo showing the associated bandgap fluorescence in solution, and (left) a multiple-layer graphene piece with defects. Bottom: (left) a carbon nanoparticle with surface defects, and (right) fluorescence emission color variations in carbon dots. (From Ref. [41])
Compared to bandgap fluorescence, defect-derived fluorescence is much brighter in the visible to near-IR region (Figure 1.6), and the emission quantum yields are comparable to those established for QDs and organic dyes. As discussed previously, surface functionalization is the most common method for introducing defects, and carbon dots, as indicated by their name, consist of core carbon nanoparticles with various surface defects created through functionalization, the resulting fluorescence consequently referred to as having defect-derived origins.\textsuperscript{41} Similarly, the fluorescence from well-functionalized GQDs could be considered as having the same origin as that from carbon dots rather than from bandgap transitions. The heteroatom doping process is considered the same as functionalization in this chapter since its effect is essentially for the introduction of $sp^3$ defects, except for in-plane or out-of-plane. For example, Briscoe \textit{et al.} prepared N-doped carbon dots from hydrothermal carbonization of chitosan,\textsuperscript{92} with the fluorescent quantum yield of the aqueous carbon dot solution calculated to be 13.4\% when excited at 360 nm, while in contrast carbon dots made from chitin and glucose are not as bright as that of chitosan. Li \textit{et al.} reported S-doped GQDs synthesized via an electrochemical approach with graphite rod and sodium toluenesulfonate,\textsuperscript{93} the as-prepared S-doped GQDs having a relatively narrow size distribution ranging from 2 to 4 nm with an average diameter of 3 nm. Based on AFM analysis, these S-doped GQDs had a typical topographic height of 0.3-1.2 nm with an average height of 0.7 nm, suggesting that most were single or bilayered. The quantum yield of these S-doped GQDs was as high as 10.6\% when excited at 380 nm, much higher than that of the GQDs.
Recently, the concept of surface states has emerged as a potential explanation for the origin of fluorescence on carbon-based quantum dots. These refer to fluorescent centers on the surface of the dots, which are predominantly composed of conjugated carbon atoms and bonded heteroatom. As the number of centers increase, the bandgap between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) is reduced, resulting in tunable fluorescence associated with this surface state. According to this surface state theory, this featureless absorption is due to the many surface states resulting in a wide distribution of different energy levels. Compared to the defect-derived origin, this theory emphasizes the importance of functionalities as well, reaffirming the complexity of the dot system, for example, the inhomogeneous and uncontrollable distribution of functionalities. However, this emphasis on the surface functionalities means that the carbon core is ignored. For carbon-based quantum dots, no matter with or without further functionalization, the carbon core is seen as the source of fluorescence since it absorbs photon and provides excited electrons. An argument against surface states is the lifetime, which should be very short like organic dye molecules and strongly dependent on different materials; however, the fluorescence decay of carbon dots is relatively similar (4~5 ns), and much longer than dye molecules, no matter how well they have been functionalized or which functionality is used.

1.3.3 Biological Toxicity
Toxicity is one of the most important properties to consider before any material is applied in a bio-related area. Carbon is a benign material serving as the backbone of life, meaning carbon-based quantum dots were expected to be nontoxic to the bio-system when they were developed. Many experiments have been conducted exploring their toxicity both in vivo and in vitro, with the results suggesting they are generally nontoxic or at least no more toxic than the surface passivation agents used.

The initial cytotoxicity evaluation of carbon dots was conducted by Yang et al. in 2009. PEG\textsubscript{1500N} functionalized carbon nanoparticles (PEG\textsubscript{1500N}-carbon dots) were introduced to two cell lines (HT-29 and MCF-7), with results based on their effect on cell proliferation, mortality, and viability (Figure 1.7) indicating they were no more toxic than that of the control group of free PEG\textsubscript{1500N}. In fact, PEG\textsubscript{1500N} at higher concentrations was somewhat more toxic to the cells than the PEG\textsubscript{1500N}-carbon dots, as the PEG polymers, similar to surfactant molecules, caused more damage to the cell membrane; however, they should not interfere with the application of PEG\textsubscript{1500N}-carbon dots since the concentration of dots used in the study were higher than those required for optical imaging of living cells. In addition, in vitro testing of PEG\textsubscript{1500N}-carbon dots in mice found that all survived the 4 weeks of the experiment without showing any abnormal symptoms even at high levels of carbon dot intake (40 mg/kg body weight), with no potential hepatic injury or kidney malfunction being observed. Furthermore, carbon dots with other surface functional groups such as PPEI-EI, polyethyleneimine (PEI) and polyallylamine (PAA) were also tested for cytotoxicity, the results indicating no or low cytotoxicity of carbon dots with different surface passivation agents; even the more
cytotoxic polymers were found to be suitable for their corresponding carbon dots to be applied in live cell imaging as long as the concentrations were kept low and/or the incubation time was short. Thus, the toxicity of carbon dots for specific bio-applications depends on the selection of appropriate nontoxic species for the surface functionalization, as their toxicity is more crucial rather than that of the carbon core in determining the overall performance of the carbon dots.¹⁰²
Figure 1.7 Results from cytotoxicity evaluations of PEG$_{1500}$N-carbon dots (black) and PEG$_{1500}$N (white). (From Ref. [101])
In addition to carbon dots, GQDs made from the exfoliation of carbon fiber via ultrasonication in acidic media were tested in vitro and in vivo. Similar to carbon dots, GQDs were found to be non-toxic in several cell lines, and they did not cause apparent toxicity in rats at different dosages (5 and 10 mg/kg) for 22 days as evidenced by blood biochemistry and hematological analyses. No severe symptoms of inflammation were observed in the liver, kidney, spleen, heart, or lung at 22 days after the administration of the GQDs.

Compared to the toxicity of traditional organic dyes or conventional QDs, the non-toxicity of carbon dots and GQDs is one of the important reasons for their rapid development. This non-toxicity is one of their significant advantages, just as important as their excellent optical properties.

1.3.4 The Ties between Carbon Dots and GQDs

While theoretically carbon dots and GQDs have different structures (0D and 2D), since GQDs are composed of small single-layer graphene fragments without functionalization by introduced groups or solvent molecules have not yet been confirmed, it is more meaningful to compare them in the real world rather than trying to differentiate them theoretically. Carbon dots and GQDs essentially have some similarities, especially because GQDs with surface functionalization are close to carbon dots in terms of both optical properties and structural characteristics, and because of the similarity between naked carbon nanoparticles and GQDs. Specifically, the functionalized GQDs involving significant sp³ carbons, which are usually characterized by Raman spectroscopy (for the
ratio between G-band and D-band,\textsuperscript{37,39,40} are more likely dominated by defect-derived fluorescence rather than conjugated $\pi$-domains. Also, the GQDs reported generally consist of multiple layers of graphene fragments\textsuperscript{40} rather than the ideal single-layer configuration. Thus, the functionalized GQDs are fundamentally no different from carbon dots. Moreover, the naked carbon nanoparticles usually emit weak fluorescence in the visible region,\textsuperscript{67} similar to that from the bandgap transitions of the GQDs. In addition, they share the same featureless absorption in the visible region, probably due to the $\pi$-plasma electrons.\textsuperscript{104} Again, the functionalization should be considered as a very important similarity between GQDs and carbon dots as the surface passivation groups help to facilitate more effective recombinations of the surface-confined elections and holes by stabilizing surface sites.

Further experimental results have demonstrated the similarities between GQDs and carbon dots as it appears that both products can be created from the same starting materials using varying experimental conditions,\textsuperscript{105,106} meaning there is a connection between these two configurations. Nie \textit{et al.} prepared two kinds of dots by refluxing diethylamine (DEA) in chloroform,\textsuperscript{105} one being blue fluorescent dots after a 1 h reaction time and the other full-color fluorescent dots after a 60 h reaction time (Figure 1.8a). From the absorption spectra (Figure 1.8b), the blue fluorescent dots were probably GQDs because of the two shoulder peaks at 228 and 282 nm, both of which might be attributed to the $\pi$-$\pi^*$ transition of C=C bonds, while full-color fluorescent dots were similar to carbon dots because of the stronger absorption in visible region.
More important, the blue fluorescent dots exhibited excitation-independent emission with a peak at 407 nm (Figure 1.8c), while the full-color fluorescent dots showed nearly continuous excitation-dependent emission extending to the near-IR region when excited in the visible region (Figure 1.8d). The full-color fluorescent dots were larger overall (2-4 nm) than the blue fluorescent (1-3 nm), probably because of the much longer reaction time. By applying proton NMR analysis, the researchers further proposed a step-wise growth reaction as the formation mechanism of the dots, starting from a simple monomer, gradually growing to cycloenes and then to larger fused rings similar to graphene fragments, a pattern which also explained the size difference between the two dots. The full-color fluorescent dots exhibited significant more C=O, C=N, and C=C groups compared to the blue fluorescent dots based on the FTIR analysis, perhaps serving as surface passivation groups on them, thus resulting in two kinds of dots.

Wang et al, who reported a synthetic route that yielded a series of GQDs with different emission colors,106 began with polycyclic aromatic hydrocarbon (PAH) molecules and applied hydrothermal fusion in alkaline solutions at low temperature, where the alkaline served as both a catalyst for low-temperature fusion and as a surface passivation agent. As the hydrothermal duration decreased from 10 to 1 h, yellow fluorescent dots, cyan fluorescent dots and blue fluorescent dots were formed, their sizes gradually decreasing from 3.8 nm to 2.9 nm and finally to 2.6 nm, respectively. The larger yellow fluorescent dots were found to have more defects than the other two dots, both characteristics perhaps associated with longer hydrothermal time. The researchers proposed a band-edge exciton-state for the florescence observed, an explanation that
worked to a degree for the cyan and blue fluorescent dots as they were likely functionalized GQDs but not as well for the yellow fluorescent dots, for which the G to D band intensity ratios were 1.03 (1.14-1.20 for cyan and blue); thus, the sp$^3$ carbon should be considered when interpreting the fluorescence mechanism, meaning that the yellow fluorescent dots could not be considered as defect-free as assumed. On the contrary, the yellow fluorescent dots were probably carbon dots, with the fluorescence resulting from defect-derived origins.
Figure 1.8 (a) Fluorescence images of blue dots (upper row) and full-color dots (lower row) in ethanol excited with the indicated wavelengths; (b) UV–visible absorption spectra of the blue dots and full-color dots in ethanol; (c, d) Fluorescence spectra of the blue dots and full-color dots in ethanol under different excitation wavelengths. (From Ref. [105])
Based on the results observed, it might be possible to gradually convert GQDs into carbon dots (or at least a similar configuration) by varying the reaction duration or temperature as usually more energy is needed for carbon dot formation because of the functionalization process on the carbon core. In addition, research has also suggested that the transition from GQDs to carbon dots can be achieved by adjusting the pH during synthesis. Hu et al. reported an approach for synthesis of a series of carbon dots with fluorescence emissions covering the entire visible spectrum (400-710 nm) by controlling the dehydration reaction.\textsuperscript{107} Citric acid (CA) or ethylene glycol (EG) was mixed with ethylenediamine endcapped polyethylenimine (M\textsubscript{n}=600, PEI-EC), this mixture then subjected to a dehydration reaction in either NaBH\textsubscript{4} or H\textsubscript{3}PO\textsubscript{4} to yield carbon dots. The different emissions were controlled by varying the amount of NaBH\textsubscript{4} or H\textsubscript{3}PO\textsubscript{4}, with the latter helping to red-shift while the former helped to blue-shift. The blue fluorescent dots were excitation-wavelength independent, while the green and red were dependent.

The size distribution of the as-prepared carbon dots was measured using dynamic light scattering (DLS), the results showing that the blue fluorescent dots were generally smaller and the red the largest. In addition, the oxygen content increased with the red-shift of the strongest, a result confirmed by carbon NMR as stronger C-O-C and weaker C=O signals were observed in red fluorescent dots than in blue and yellow fluorescent dots. From a structural perspective, the carboxyl and carbonyl groups introduced by the H\textsubscript{3}PO\textsubscript{4} during the dehydration process probably served as surface passivation groups in addition to the possible passivation by the amino groups in the starting materials, resulting in fluorescent carbon dots in the visible region. Due to the strong reduction of
NaBH₄, the carbon dots formed might go through a process of defunctionalization as confirmed by their much lower oxygen content; thus, the blue fluorescent dots were probably poorly functionalized carbon dots or close to naked carbon nanoparticles, both of which are very similar to GQDs.

The connection between GQDs and carbon dots is a key to better understanding carbon-based quantum dots. Recent literature usually considers them separately or does not compare them directly as there is not enough information available for a conclusion to be made. In addition, many papers randomly borrow the “name” without a deep understanding of the product being investigated, consequently lending to the confusion in the field.³³,¹⁰⁸-¹¹⁰ The analysis presented here points to a suggestion of a connection between GQDs and carbon dots based on the available results, primarily to draw attention to it. GQDs and carbon dots share several advantages as benign fluorescent materials, and a comprehensive comparison suggests other similarities as well. Furthermore it is possible to convert one to the other under special conditions.

1.4 Applications

1.4.1 Optical Bioimaging

As benign fluorescent nanomaterials, both GQDs and carbon dots have been applied as bright probes in vitro and in vivo,¹¹¹-¹²⁶ their most significant advantage being their optical properties which are comparable to conventional QDs but with no obvious toxicity. Although there are still challenges such as relative low emission in the red
towards the red/near-IR region and lack of uniformity in the size and distribution of the surface passivation groups, considerable attention has been focused on this field, the results providing convincing evidence supporting the need for further development.\textsuperscript{71}

1.4.1.1 Cell Imaging

As originally demonstrated by Sun et al.,\textsuperscript{28} carbon dots with surfaces passivated by PEG\textsubscript{1500N} were applied to successfully stain \textit{E. coli} ATCC 25922 and Caco-2 cells, confocal microscopy imaging showing that the carbon dots were readily taken up by the cells, mostly residing in the cytoplasm, with only minor penetration into the cell nucleus. Similar subsequent experiments have been conducted since then, all demonstrating carbon dots as bright nano-probes \textit{in vitro}. Song et al. prepared carbon dots from a popular antibiotic, aminosalicylic acid, (ASA) for cell imaging.\textsuperscript{111} After incubating with carbon dots for 4 h, the confocal laser fluorescence microscope (CLSM) imaging of H1299 cells indicated intense green, yellow, and red emissions under 405, 488 and 542 nm excitations, respectively. Liu et al. synthesized carbon dots using rose-heart radish as the carbon precursor via a simple hydrothermal treatment and applied in SiHa cells.\textsuperscript{112} Upon incubation with the SiHa cells (B16-F10) for 3 h, the carbon dots were taken up by the cells, with the observation of meaningful blue, green, and red fluorescence emissions corresponding to different excitation wavelengths. Zhang et al. reported the synthesis of aqueous compatible carbon dots using one-pot hydrothermal processing of nanodiamond.\textsuperscript{113} The carbon dots were internalized into the NIH-3T3 cells upon incubation, with green and yellow fluorescence emissions at 405 nm and 458 nm
excitations, respectively, found in the cell cytoplasm. Wang et al. developed a turn-on nanoprobe for selenol recognition in living cells based on fluorescent carbon dots prepared from $m$-aminophenol via a solvothermal process.\textsuperscript{114} The synthesized carbon dots were strongly fluorescent upon incubation with selenocysteine (Sec) due to a nucleophilic substitution process, resulting in amines as passivation groups on the surface of the carbon dots. These results indicated that the synthesized carbon dots were cell permeable and feasible for imaging endogenous Sec inside live cells.

More recently, carbon dots with their surfaces passivated by simple molecules instead of large polymers were prepared and applied in the fluorescent labeling of stem cells.\textsuperscript{115} The use of simple molecules to functionalize carbon dots is an advantage as their smaller size makes them more efficient for renal clearance than the larger polymers.\textsuperscript{116} Liu et al. exploited 2,2’-(ethylenedioxy)bis(ethylamine)-functionalized carbon dots for the labeling of mesenchymal stem cells (MSCs).\textsuperscript{115} The carbon dots were found in the cell membrane and cytoplasm for both live and fixed cells, though the labeling efficiency was significantly lower in the live cells (Figure 1.9). This difference was attributed to the cationic surface character of the carbon dots at the biological pH, which might be less favorable for efficient uptake by the live cells. According to their results, the surface functionalities on carbon dots may play a significant role in determining the cell labeling efficiency.
Figure 1.9 Left: Merged (fluorescence + bright-field) images of the live stem cells labeled with the EDA-carbon dots. Right: Merged (fluorescence + bright-field) images of the fixed stem cells labeled with the EDA-carbon dots. (From Ref. [115])
GQDs have been applied to the fluorescence imaging of cells and tissues in a conceptually similar fashion to that of the carbon dots highlighted above. For example, Zhao et al. reported that GQDs prepared from petroleum asphaltene could be effectively uptaken by HeLa cells, exhibiting green fluorescence excited by a 488 nm laser.\textsuperscript{118} The results observed suggested that GQDs were endocytosed into the HeLa cells and were evenly dispersed in the cytoplasm rather than captured by the nucleus. Hai et al. prepared boron-doped GQDs (B-GQDs) via a one-pot acid-free microwave approach for imaging in HeLa cells.\textsuperscript{119} After incubation with a DMEM culture medium containing B-GQDs for 12 h, the HeLa cells were found to exhibit bright blue fluorescence with excitation at 330 nm. Because of their limited cytotoxicity, B-GQDs have been suggested as candidates for bio-labelling agents for living cells. Roy et al. reported a novel method utilizing GQDs derived from plant leaves to label both normal cells (MCF-10A cells) and cancer cells (HeLa and MCF-7 cells).\textsuperscript{120} After treatment with GQDs, green and red cell images were observed when excited with an argon laser light (488 nm). Peng et al. prepared GQDs from carbon fibers to image human breast cancer cell T47D.\textsuperscript{121} After incubation for 4 h, agglomerated green GQDs were found surrounding each cell nucleus. Sun et al. used chemically and photochemically reduced GQDs to label A549 cells,\textsuperscript{122} with blue fluorescence being observed around the cell nucleus at 380 nm excitation.

1.4.1.2 In Vivo Imaging

After the in vitro studies indicated that carbon dots and GQDs were as viable as QDs and because of their nontoxic nature, research soon focused on their potential in in
in vivo imaging, the results confirming their feasibility, the first step towards further clinical application. The first in vivo evaluation of carbon dots was reported by Yang et al., in which PEG$_{1500N}$ functionalized carbon dots were subcutaneously injected into mice to monitor their migration toward the axillary lymph node. Fluorescent images at different excitation wavelengths were obtained, and the contrast from both the green and red emissions was sufficient for imaging (Figure 1.10). The results suggested that carbon dots were primarily excreted via urine with a very low accumulation level in the kidney. Similar results were reported by Wu et al. using carbon dots prepared from honey in a mouse model, their results showing a high contrast enhancement in auxiliary lymph nodes. As pointed out by these researchers, the rapid lymphatic transport of these dots might be a valuable property, offering greater convenience and reduced procedural expense, as well as improved surgical advantage as the patient is positioned on the table for easier resection.
Figure 1.10 Top: Interdermal injection of PEG$_{1500N}$-carbon dots. Insets are the dissected (in the circled area) axillary lymph node (LN). Bottom: The *in vivo* imaging of KB tumor bearing mice after intravenous injection of GQDs. (From Ref. [123])
Carbon dots are generally fluorescent in the green rather than in the red/near-IR, but the latter is more favorable for in vivo imaging because of its low background noise and better tissue transmittance. As a result, research efforts have focused on extending the emission spectral range of carbon dots to red/near-IR. In the study conducted by Huang et al., carbon dots were linked with fluorescence dye ZW800 to extend their spectral range to this region. The resulting dots were tracked in vivo for their biodistribution, excretion, and passive tumor uptake by using both near-IR fluorescence and positron emission tomography imaging techniques. The carbon dots were efficiently and rapidly excreted from the body after injection through different routes, with some being found in the liver, spleen, and lungs within an hour post intravenous injection, and very bright fluorescence was observed in kidneys, with urine excretion also being confirmed. All injection pathways led to meaningful tumor uptakes. In a related study, Huang et al. attached fluorescence dye Ce6 to carbon dots to impart red fluorescence emissions through FRET, finding that the conjugate of Ce6-carbon dots could be excited at 430 nm for red fluorescent emissions (668 nm). After intravenous injection, the accumulation of the dots in the tumor was detected, and the laser excitation of the probes in the mice significantly suppressed the tumor growth.

The application of GQDs in vivo imaging has also been reported. For example, Nurunnabi et al. prepared carboxylated GQDs for imaging in vivo, introducing these synthesized GQDs into nude mice through intravenous injection, with fluorescent emissions observed in the entire body 30 min post-injection (Figure 1.10). The fluorescence intensities remained for approximately 8 h, decreasing slowly until
becoming very weak after 16 h. The results provided substantial evidence of the safety of GQDs for biomedical application.\textsuperscript{103}

1.4.1.3 Multiphoton Imaging

Up-conversion fluorescence is a phenomenon in which the sequential absorption of two or more photons leads to the emission of light at a shorter wavelength than the excitation wavelength.\textsuperscript{127} Compared to normal fluorescence, up-conversion is more favorable in vivo imaging since longer excitation wavelengths (especially in the near-IR region) are usually preferred due to reduced photon-induced tissue damage, deep-tissue penetration, and reduced background auto-fluorescence.\textsuperscript{128} Both GQDs and carbon dots are able to afford up-conversion fluorescence, the efficiency of which is much higher than that of conventional fluorophores.\textsuperscript{129-132}

Cao et al. reported carbon dots prepared via deliberate functionalization of carbon nanoparticles with PPEI-EI (with EI fraction ~20%), which were applied for cell imaging using two-photon luminescence microscopy.\textsuperscript{129} After incubation with PPEI-EI-carbon dots, the MCF-7 cells became brightly illuminated when imaged using a fluorescence microscope with excitation by 800 nm laser pulses. Under this excitation using a femtosecond pulsed laser, the estimated two-photon absorption cross-section was $39000 \pm 5000$ GM (Goeppert-Mayer unit, with $1 \text{GM} = 10^{-50} \text{cm}^4 \text{s/photon}$), much higher than the conventional CdSe QDs at 780-10300 GM and comparable to the core-shell QDs CdSe/ZnS at 50000 GM. Liu et al. reported dimethylamine functionalized GQDs (NGQDs) as efficient two-photon fluorescent probes for cellular and deep-tissue
The NGQDs were prepared by using GO as the graphene resource and dimethylamine as the passivation agent via solvothermal treatment. The estimated two-photon absorption cross-section reached 48000 GM, a result which is even better than carbon dots and far surpassing that of organic dyes. In addition, the large imaging penetration depth of 1800 μm achieved by the synthesized NGQDs in tissue phantom significantly extended the fundamental imaging depth limit of two-photon microscopy.

1.4.2 Photocatalytic Energy Conversion

Because of the recent increase in the amount of CO₂ in the atmosphere and its impact on our environment, much research has focused on investigating ways for reducing it, one approach involving the photocatalytic conversion of CO₂ into hydrocarbon fuels. Not only would it address the environment concerns but it would also regenerate an energy source by harvesting inexhaustible solar power. Semiconductor nanomaterials such as TiO₂ and CdS have been employed as photocatalysts in such relevant reactions. However, their wide-bandgap transitions have limited their absorption within the UV region, meaning the utilization of the entire solar spectrum is very inefficient; in addition, the high energy of UV may adversely damage small fuel compounds. On the other hand, carbon-based quantum dots, which are conceptually similar to semiconductor nanomaterials, have demonstrated the capability of harvesting UV/visible light, extending into the near-IR region, and the photoinduced redox processes observed in carbon-based quantum dots suggest they are an excellent opportunity for catalytic energy conversion reactions. Some representative studies are
highlighted here in which both GQDs and carbon dots were used in photocatalytic CO₂ conversion. ¹³⁷,¹³⁸

Li et al. coated PEG₁₅₀₀N functionalized carbon dots with gold or platinum through simple solution-phase photolysis,¹³⁷ for the photocatalytically enhanced reduction reaction illustrated in Figure 1.11. The coated carbon dots were then used as photocatalysts for the reduction of CO₂, with formic acid being detected. The estimated quantum yields for this reduction reaction were approximately 0.3%, which were an order of magnitude higher compared to TiO₂ nanoparticles as photocatalysts with UV light irradiation using the same procedure. It was also found that the same carbon dots could be used as photocatalysts for H₂ generation from water. In a further study, Sahu et al. demonstrated that gold-coated PEG₁₅₀₀N-carbon dots could also act as photocatalysts to convert CO₂ into acetic acid under high pressure, a reduction requiring more electrons than for formic acid,¹³⁸ with the calculated efficiency being much higher than for semiconductor nanoparticles.
Figure 1.11 Illustration of CO$_2$ conversion with metal doped PEG$_{1500N}$-carbon dots as photocatalysts under normal (a) and high pressure (b). (From Ref. [137] and [138])
On the other hand, studies exploring GQDs as photocatalysts for CO$_2$ are limited, probably because their photoexcited state redox properties are less robust, as the CO$_2$ conversion represents one of the most challenging photocatalytic reactions.$^{21}$ This analysis is supported by the fact that “naked” carbon nanoparticles, namely those without any deliberate surface functionalization by organic or other molecules, were applied as photocatalysts using a similar procedure for CO$_2$ conversion as that for the PEG$_{1500N}$-carbon dots, but the performance was generally poor.$^{139}$ As demonstrated previously, again the “naked” carbon nanoparticles and GQDs are conceptually similar, especially since the as-prepared GQDs usually consist of multi-layer graphene fragments. Mechanistically, the photocatalytic performance might be associated with the relative stability of the photogenerated redox species in the carbon-based quantum dots, a situation which is probably enhanced substantially by the surface passivation agents inhibiting the quenching effects.$^{21}$

1.5 Conclusion

Carbon dots and GQDs as novel carbon-based quantum dots have emerged as a new class of fluorescent nanomaterials with comparable optical properties with organic dyes and conventional QDs.$^{20-27}$ Recent advances in this field are discussed in this chapter, its goal being a systematic comparison between carbon dots and GQDs focusing on their synthetic methods, properties, and applications. Based on the results, the well-defined carbon dots have gradually become understood, especially because of the recent evidence of the surface attached passivation groups obtained through NMR
characterization.\textsuperscript{30,32} On the other hand, the perfect GQDs, which are supposed to be single-layer graphene fragments without any functionalization, remain a mystery, and in most cases the available GQDs consist of multi-layer of graphene fragments with defects on surface. Thus, these GQDs are actually very similar to carbon dots in terms of their chemical and physical properties, and consequently are not very different from an application perspective. Furthermore, some experimental results have already demonstrated it might be possible to achieve a conversion between likely GQDs and carbon dots from the same precursors, further support of the similarity between the two. Practically speaking, carbon dots have the advantage in the visible to the near-IR region, reducing interest in theoretical GQDs. The current challenge is the synthesis of more uniform carbon dots and GQDs as the existing dots are inhomogeneous in terms of size and surface passivation distribution, among other characteristics, which definitely impact their viability.
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CHAPTER TWO
FUNCTIONALIZATION OF CARBON NANOPARTICLES AND
DEFUNCTIONALIZATION - TOWARDS STRUCTURAL AND MECHANISTIC
ELUCIDATION OF CARBON "QUANTUM" DOTS

This work has been published as:

2.1 Introduction

Semiconductor nanocrystals, commonly referred to as quantum dots (QDs), exhibit strong size-dependence of optical properties when the nanocrystal sizes are smaller than the Bohr exciton radius.\(^1\)\(^-\)\(^6\) Compared with organic dyes and fluorescent proteins, QDs are known for their strong optical absorption, high fluorescence emission quantum yields, large Stokes shifts, and excellent resistance to photobleaching.\(^7\),\(^8\) However, high-performance QDs are mostly those containing heavy metals such as cadmium and lead, whose known toxicity has raised significant concerns and may ultimately prove prohibitive for many desired applications.\(^9\),\(^10\) Much effort has been made in the search for QD-like optical nanomaterials derived from benign elements, among which carbon-based QDs,\(^11\),\(^12\) especially carbon dots,\(^13\) have attracted widespread attention. In fact, carbon dots research now represents a rapidly advancing and expanding field, as reflected by the large number of recent publications in the literature.\(^11\),\(^14\)\(^-\)\(^20\)

Carbon dots are generally defined as small carbon nanoparticles with various
As a new class of QD-like fluorescent nanomaterials, carbon dots have been shown for possessing many advantageous properties amenable to their serving as bright optical probes for a variety of imaging and sensing applications, including readily aqueous soluble, high optical performance and non-blinking, physicochemical and photochemical stabilities, and benign and nontoxic to cells and in animals according to available cytotoxicity and in vivo toxicity assays. The surface passivation in carbon dots apparently plays a critical role in their optical performance, especially the fluorescence brightness and associated properties. While fluorescence emissions have been observed from seemingly "naked" carbon nanoparticles, including those of a more defined graphitic structure or from partially reduced graphene oxides that are commonly referred to as "graphene quantum dots", in aqueous or other suspensions, the emission intensities are relatively low in general and mostly centered in the UV/blue spectral region. The surface passivation has been shown to substantially enhance the fluorescence performance, with the most effective being chemical functionalization of the carbon nanoparticle surface by organic molecules (Figure 2.1). For example, carbon dots with the oligomeric poly(ethylene glycol) diamine (PEG$_{1500}$N, Figure 2.1) for surface functionalization exhibited fluorescence quantum yields of up to 60%. 


Figure 2.1 A cartoon illustration on carbon dot, which is generally a small carbon nanoparticle core with attached and strongly adsorbed surface passivation molecules (a configuration similar to a soft corona).
More recently, smaller amino molecules such as 2,2’-(ethylenedioxy)bis(ethylamine) (EDA, molecular weight 148) were used successfully in the functionalization of carbon nanoparticles for brightly fluorescent carbon dots, which are structurally ultra-compact and of a relatively simpler surface passivation layer (Figure 2.1).\textsuperscript{35} As a result, more traditional solution-phase characterization techniques such as proton and carbon-13 NMR methods could be applied to the structural elucidation of the carbon dots. In the study reported here, we used another small amino molecule, 3-ethoxypropylamine (EPA, molecular weight 103, Figure 2.1), for the functionalization of small carbon nanoparticles to obtain carbon dots of significantly higher fluorescence performance than that of EDA-carbon dots. The similarly ultra-compact EPA-carbon dots exhibited enhanced optical absorption that could be correlated with the observed high fluorescence quantum yields, and the correlation was investigated systematically via the defunctionalization of the EPA-carbon dots. The combination of functionalization and defunctionalization experiments and outcomes provided valuable insight into the critical role of surface passivation in carbon dots, contributing to an improved mechanistic understanding of carbon dots for their optical properties and other related functions.

### 2.2 Experimental Section

**Materials.** Carbon nano-powders (US1074) were supplied by US Research Nanomaterials, Inc. 3-Ethoxypropylamine (EPA) was purchased from TCI, thionyl chloride (>99%) from Alfa Aesar, and nitric acid and sodium hydroxide from Fisher Scientific. Deuterated water for NMR experiments was obtained from Cambridge Isotope
Laboratories, and dialysis membrane tubing (molecular weight cutoff 100~500) from Spectrum Laboratories. Water was deionized and purified by being passed through a Labconco WaterPros water purification system.

**Measurements.** UV/vis absorption spectra were recorded on a Shimadzu UV2501-PC spectrophotometer. Fluorescence spectra were acquired on a Jobin-Yvon emission spectrometer equipped with a 450 W xenon source, Gemini-180 excitation and Tirax-550 emission monochromators, and a photon counting detector (Hamamatsu R928P PMT at 950 V). 9,10-Bis(phenylethynyl)-anthracene in cyclohexane was used as a standard in the determination of fluorescence quantum yields by the relative method (matching the absorbance at the excitation wavelength between the sample and standard solutions and comparing their corresponding integrated total fluorescence intensities). Fluorescence decays were measured in terms of the time-correlated single photon counting (TCSPC) technique on a Horiba Ultima Extreme spectrometer. The spectrometer is equipped with a SuperK Extreme supercontinuum laser source operated at 3.894 MHz repetition rate, TDM-800 excitation and TDM-1200 emission monochromators, a R3809-50 MCP-PMT detector operated at 3.0 KV in a thermoelectrically cooled housing, and FluoroHub A+ timing electronics. Analyses of the decay curves were performed by using the Horiba Das6 fluorescence decay analysis software. NMR measurements were carried out on a Bruker Advance 500 NMR spectrometer. Atomic force microscopy (AFM) images were acquired in the acoustic AC mode on a Molecular Imaging PicoPlus AFM system equipped with a multipurpose scanner and a NanoWorld point probe NCH sensor. The height profile analysis was
assisted by using the SjPIP software distributed by Image Metrology. Transmission electron microscopy (TEM) images were obtained on a Hitachi H-9500 high-resolution TEM system.

**Carbon Dots.** Small carbon nanoparticles were harvested from the commercially acquired carbon nano-powders in procedures similar to those reported previously.\textsuperscript{35} In a typical experiment, a sample of carbon nano-powders (2 g) was refluxed in aqueous nitric acid (8 M, 200 mL) for 48 h. The reaction mixture was cooled to room temperature, and centrifuged at 1,000 g to discard the supernatant. The residue was re-dispersed in deionized water, dialyzed in a membrane tubing (molecular weight cut-off ~500) against fresh water for 48 h, and then centrifuged at 1,000 g to retain the supernatant. Upon the removal of water, small carbon nanoparticles were recovered for their being used in the functionalization reaction.

The carbon nanoparticles obtained above were dispersed in neat thionyl chloride and then refluxed for 12 h. The excess thionyl chloride was removed, and the treated sample (50 mg) was mixed well with 3-ethoxypropylamine (EPA, 1 g) in a round-bottom flask, heated to 110 °C, and vigorously stirred under nitrogen protection for 72 h. The reaction mixture was cooled to room temperature, dispersed in water, and then centrifuged at 20,000 g to retain the supernatant, followed by dialysis in a membrane tubing (molecular weight cut-off ~500) against fresh water to yield the EPA-carbon dots in an aqueous solution. $^1$H NMR (500 MHz, D$_2$O) $\delta$ 3.46 (m, br), 2.93 (m, br), 1.79 (m, br), 1.04 (m, br) ppm; $^{13}$C NMR (125 MHz, D$_2$O) $\delta$ 67.48, 66.42, 37.53, 26.85, 14.08 ppm.
Some of the EPA-carbon dots were doped with a small amount of gold via photolysis for the TEM imaging purpose. In a typical experiment, an aqueous solution of the EPA-carbon dots was mixed with an aqueous solution of HAuCl₄, and the mixture was irradiated with visible light. The doping level was kept very low, as monitored in terms of the initial emergence of the gold plasmon absorption band.

The progressive defunctionalization of the EPA-carbon dots was carried out under base-catalyzed hydrolysis conditions. Experimentally, to an aqueous solution of the EPA-carbon dots (0.5 mg/mL, 20 mL) was added sodium hydroxide (0.4 g, 0.5 M), and the solution was heated to 80 °C. After 4 h, the temperature was increased to 100 °C, along with an increase of the base concentration (0.75 M). After another 4 h, the temperature was further increased to 120 °C, so was the base concentration (1.0 M), and the reaction was allowed to proceed for 4 more hours. Finally, the temperature was increased to 130 °C, and the mixture was reacted for 8 h. During the reaction period (a total of 20 h), a portion of the reaction mixture was collected in 2 h intervals for spectroscopic and other characterizations.

2.3 Results and Discussion

Commercially acquired carbon nano-powders were refluxed in aqueous nitric acid for purification and also for the introduction of oxygen-containing functionalities such as carboxylic acids on the particle surface, followed by dialysis and centrifugation to harvest mostly smaller carbon nanoparticles in an aqueous suspension. The carbon nanoparticles thus obtained were functionalized with EPA molecules for EPA-carbon
dots in a typical amidation reaction scheme involving the use of thionyl chloride for the acylation of the particle surface-bound carboxylic acid moieties for amide linkages with EPA molecules. Free EPA and other small molecular impurities were removed via dialysis to obtain the EPA-carbon dots sample as a colored aqueous solution (Figure 2.2).
Figure 2.2 The absorption spectrum of the EPA-carbon dots in aqueous solution (——) is compared with those of the aqueous suspended carbon nanoparticles (- .. -) and the PEG1500N-carbon dots (- - - -).25 The photos are for concentrated (left) and dilute (right) aqueous solutions of the EPA-carbon dots. The other inset shows a comparison of two spectra corresponding to the extra absorption shoulder, obtained by subtracting the spectrum of the carbon nanoparticles (- - - -) or the final defunctionalized sample (——) from that of the EPA-carbon dots.
For atomic force microscopy (AFM) analyses, aqueous solution of the EPA-carbon dots was diluted, from which a few drops were deposited onto a piece of mica, followed by the removal of water via evaporation. As shown in Figure 2.3, the EPA-carbon dots are relatively well defined, with the height analysis results of multiple AFM images suggesting an average dot size of 4.5 nm and size distribution standard deviation of 0.9 nm in diameter. For the transmission electron microscopy (TEM) imaging, the EPA-carbon dots in aqueous solution were coated with a small amount of gold in simple photolysis to increase the imaging contrast. Shown in Figure 3 is a representative TEM image, where the dot profiles are generally comparable with those found in the AFM analysis. According to the microscopy results, the EPA-carbon dots are similarly compact in comparison with the previously reported EDA-carbon dots.
Figure 2.3 Results from AFM (top) and TEM (bottom) imaging of the EPA-carbon dots.
The UV/vis absorption spectrum of the EPA-carbon dots in aqueous solution is shown in Figure 2. Generally, the absorption of carbon dots is due primarily to the core carbon nanoparticles (Figure 2.1), with the particle surface functionalization by colorless molecules playing a rather minor role. For the EPA functionalization, however, there are apparently significant new features in the absorption spectrum of EPA-carbon dots, specifically the shoulder in the blue region that is in addition to the absorption of the core carbon nanoparticles (Figure 2.2). Similar absorption features were previously found in carbon dots with the oligomeric poly(ethylene glycol) diamine (PEG$_{1500}$) functionalization, as also shown in Figure 2 for comparison.$^{25}$ These significant absorption spectral changes suggest that upon the surface functionalization of carbon nanoparticles with the selected organic molecules, there could be significant effect associated with the functionalization to alter the electronic transitions of the core carbon nanoparticles in the resulting carbon dots, corresponding to the enhanced absorption in the blue as reflected by the additional absorption shoulder (Figure 2.2). Since the mode of functionalization was the formation of amide linkages between the carbon nanoparticle surface-bound carboxylic acid moieties and EPA molecules,$^{20,25}$ as targeted by the amidation reaction conditions for the functionalization, the effect on the optical absorption of the resulting carbon dots seems unusually significant, for which a mechanistic elucidation remains a challenge. Apparently, the altered electronic transitions reflected in the observed absorption spectra were also consequential to the observed fluorescence emission properties.
Fluorescence spectra of the EPA-carbon dots in aqueous solution are dependent on excitation wavelengths (Figure 2.4), similar to those of carbon dots with other surface functional molecules. Correspondingly, the observed fluorescence quantum yields, determined in reference to 9,10-bis(phenylethynyl)anthracene as a standard (quantum yield of unity, calibrated against the quinine sulfate standard), are also excitation wavelength dependent (Figure 2.4). The peak fluorescence quantum yield was found at around 440 nm excitation, coinciding with the shoulder in the absorption spectrum (Figure 2.4). Therefore, the above discussed enhancement in optical transitions due to the specific effect associated with the carbon nanoparticle surface functionalization by EPA molecules is apparently correlated with significantly brighter fluorescence emissions, likely sharing the same mechanistic origin. It is probably no coincidence that the PEG_{1500N}-carbon dots with the enhanced optical absorption (a similar absorption shoulder in the blue spectral region, Figure 2.2) also exhibited significantly higher fluorescence quantum yields when excited into the absorption shoulder around 440 nm. In terms of photophysical principles, fluorescence quantum yield ($\Phi_F$) is a function of lifetime ($\tau_F$) and radiative rate constant ($k_F$) of the emissive excited state, $\Phi_F = k_F \tau_F$, where $k_F$ is proportional to the transition probabilities or approximately the optical absorptivities. Therefore, the absorption and fluorescence emission results presented above suggest that the observed higher fluorescence quantum yields when excited into the additional shoulder of the absorption spectrum (Figure 2.2) may have been contributed in a significant part by larger $k_F$ values, again reflecting the enhanced optical transitions (specifically radiative transitions from the corresponding emissive excited states to the
Such an assessment was supported by the results from fluorescence decay measurements.
Figure 2.4 Absorption (ABS) and fluorescence (FLSC) spectra at different excitation wavelengths (from left to right: 400 nm, 440 nm, 480 nm, 520 nm, 560 nm, and 600 nm) of the EPA-carbon dots in aqueous solution. Also shown is the excitation wavelength dependence of the observed fluorescence quantum yields ($\bigcirc$).
The time-correlated single photon counting (TCSPC) technique was employed for determining the fluorescence decay profiles of the EPA-carbon dots in aqueous solution at different excitation wavelengths. Shown in Figure 5 is the decay curve at 440 nm excitation, and those at other excitation wavelengths are only slightly different. The decays could not be deconvoluted quantitatively with a mono-exponential function, but reasonably well with a bi-exponential function (Table 2.1). Despite the good deconvolution fits, however, it would be an oversimplification to assume only two emission contributions at all of these different excitation wavelengths. The excited states and processes in carbon dots are likely more complicated, so that the good deconvolution fits were probably just a result of phenomenological bi-exponential averaging of the likely multi-component decays. A further averaging was made by using the pre-exponential factors ($A_1$ and $A_2$) and lifetimes ($\tau_{F1}$ and $\tau_{F2}$) from the deconvolution fits, $<\tau_F> = (A_1\tau_{F1}^2 + A_2\tau_{F2}^2)/(A_1\tau_{F1} + A_2\tau_{F2})$, and the average fluorescence lifetime $<\tau>$ values thus calculated are also shown in Table 1. These averages provide a rough estimate of the relative time constants for the observed decay curves at different excitation wavelengths. While still somewhat excitation wavelength dependent (Table 2.1), their variations are not as pronounced as those in the corresponding fluorescence quantum yields (Figure 2.4). The results again suggest that some of the changes in observed fluorescence quantum yields over different excitation wavelengths are due to similar changes in the fluorescence radiative rate constant ($k_F$, Table 2.1), which are correlated with the changes in optical absorptivities.\textsuperscript{38}
Figure 2.5 Observed fluorescence decays of the EPA-carbon dots (red) and the four samples with different degrees of defunctionalization listed in Table 2 (D₁: blue, D₂: magenta, D₃: olive, and D₄: navy), all at 440 nm excitation and the monitored emission centered at 510 nm.
Table 2.1 Results from the Deconvolution of the Observed Fluorescence Decays

\[
\langle \tau_F \rangle = \frac{A_1 \tau_{F1}^2 + A_2 \tau_{F2}^2}{A_1 \tau_{F1} + A_2 \tau_{F2}} \quad (\text{see ref. 37})
\]

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<th>(\lambda_{EM} ) (nm)</th>
<th>(\tau_F ) (ns)</th>
<th>(\chi^2)</th>
<th>(A_1) (%)</th>
<th>(A_2) (%)</th>
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<th>(\tau_{F2}) (ns)</th>
<th>(\chi^2)</th>
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<td>93(1)</td>
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<td>94(2)</td>
<td>0.5(0.3)</td>
<td>4.1(0.5)</td>
<td>1.0-1.1</td>
<td>4.1(0.4)</td>
</tr>
</tbody>
</table>

\(^a\) The average fluorescence lifetime \(\langle \tau_F \rangle = (A_1 \tau_{F1}^2 + A_2 \tau_{F2}^2)/(A_1 \tau_{F1} + A_2 \tau_{F2})\) (see ref. 37).
On the structure of carbon dots (Figure 2.1), the amidation reaction targets specifically the carboxylic acid moieties on the carbon nanoparticle surface, which are known as being generated in the oxidative acid treatment of the nanoparticles. The reaction conditions are much too mild for anything other than the formation of amide linkages, far from those required for any structural modification or heteroatom replacement of the surface carbons in the carbon nanoparticles. In this regard, the issue for structural analyses was whether the amidation reaction did result in the attachment of EPA molecules to the carbon nanoparticles. Thus, the EPA-carbon dots were characterized in solution by using proton and carbon-13 NMR techniques. Shown in Figure 6 are the proton NMR results of the EPA-carbon dots in deuterated water. The signals for all protons in the dot-attached EPA moieties are significantly broadened in comparison with those in free EPA molecules, where the broadening effect is consistent with the attachment of EPA to carbon nanoparticles as larger species of lower mobility (thus slower isotropic averaging), and also the inhomogeneous distribution of sites for the functionalization on the carbon nanoparticle surface. Similar effect is established in the literature for other surface functionalization of nanoscale species, such as in chemically functionalized carbon nanotubes and inorganic nanoparticles. In addition to the signal broadening, the changes in chemical shift are also consistent with the attachment of EPA to carbon nanoparticles. The α proton signal is downfield-shifted to 2.93 ppm (vs 2.56 ppm in free EPA), which may be attributed to de-shielding effect as a result of the amidation. The β proton signal is also downfield-shifted, but to a lesser extent, while the other proton signals are unchanged in chemical shifts (Figure 2.6). The
signal integrations are largely unchanged from free EPA molecules to their attachment to carbon nanoparticles, suggesting that other than the functionalization affecting the chemical environments of the α and β protons, the EPA molecular structure is intact, as one would expect. The carbon-13 NMR results are generally consistent with the proton NMR results, with also the signal broadening effect. As often observed\textsuperscript{43-45}, the shifts in carbon-13 NMR signals due to chemical environment changes following the functionalization are not as pronounced as those in proton NMR signals.
Figure 2.6 Proton NMR spectra of free EPA molecules (top) and the EPA-carbon dots (bottom) in deuterated water.
Figure 2.7 Carbon-13 NMR spectra of free EPA molecules (top) and the EPA-carbon dots (bottom) in deuterated water.
The NMR and other results described above are in support of or consistent with the dot structure of a small carbon nanoparticle core functionalized by EPA molecules (Figure 2.1). Further structural elucidation was pursued by chemically defunctionalizing the EPA-carbon dots to gradually remove the attached EPA molecules from the surface passivation layer, coupled with the monitoring of progressive spectroscopic changes in the samples associated with different levels of defunctionalization.

Since the functionalization in the synthesis of the EPA-carbon dots was by amidation chemistry, the chemical defunctionalization was carried out under base-catalyzed hydrolysis reaction conditions. In order to be able to monitor the gradual defunctionalization process, the hydrolysis reaction conditions were controlled by starting at a lower base concentration and also a lower reaction temperature, and then increasing stepwise both the base concentration and reaction temperature until the formation of substantial precipitation due to nearly completely defunctionalized carbon nanoparticles. As the reaction progressed, a portion of the reaction mixture was collected at different reaction time points for spectroscopic analyses. Proton NMR results of the sample solutions exhibited no significant changes, which should be expected as the defunctionalization merely decreased the population of the carbon nanoparticle surface-bound EPA species. The UV/vis absorption spectra for the samples collected at different reaction time points are shown in Figure 8. The absorption shoulder in the blue spectral region obviously decreased progressively, and largely diminished at the conclusion of the reaction, with the resulting absorption spectrum similar to that of the "naked" carbon nanoparticles before the functionalization (Figure 2.8). These results serve to confirm that
the enhanced optical transitions reflected by the additional absorption shoulder (Figure 2) are due to the EPA functionalization of carbon nanoparticles.
Figure 2.8 Absorption and fluorescence (FLSC, 440 excitation, in the inset) spectra of the EPA-carbon dots (—) and the four samples with different degrees of defunctionalization listed in Table 2 (D1: — —, D2: -.-.-, D3: -.-.-, and D4: - - -). Also shown in the inset are the changes in fluorescence quantum yield with the defunctionalization at 400 nm (○) and 440 nm (□) excitations.
Fluorescence spectra of the same samples collected at different defunctionalization reaction points are also shown in Figure 8. The spectral profiles are similar between different samples, but the emission intensities are progressively lower, so are the observed fluorescence quantum yields (Figure 2.8). However, the decrease in yields at 440 nm excitation is relatively more rapid than that at 400 nm excitation, likely due to the fact that upon defunctionalization the absorption shoulder responsible for the enhanced fluorescence emissions in the original EPA-carbon dots disappears quickly (Figure 2.8). For the sample collected at the end of the defunctionalization reaction, namely the remaining solution without the precipitate, the fluorescence quantum yields are around 4% for both 400 nm and 440 nm excitations, getting close to those of suspended carbon nanoparticles without deliberate surface functionalization by organic molecules.\textsuperscript{48-55} Thus, these results provide unambiguous evidence for the notion that the surface functionalization of carbon nanoparticles by otherwise colorless organic molecules like EPA is responsible for the observed bright and colorful fluorescence emissions of carbon dots, reaffirming the definition of carbon dots as surface-passivated (functionalized) small carbon nanoparticles.\textsuperscript{12,13,20} More specifically with the EPA functionalization, the fluorescence emissions can be further enhanced when the dots are excited into the additional absorption shoulder in the blue spectral region (Figure 2.2).

The samples with different degrees of defunctionalization were also evaluated by fluorescence decay measurements. As compared in Figure 2.5, there are no major changes in the fluorescence decay curves between the samples, suggesting that the dynamic processes in the emissive excited states are decoupled from the dramatic
decreases in fluorescence quantum yields upon defunctionalization (Figure 2.8). The
decay curves could again be deconvoluted well with a bi-exponential function, and the
results are shown in Table 2. The averaging of the two lifetime values allows the
calculation of fluorescence radiative rate constants, \( k_F = \Phi_F/\tau_F \), where \( \Phi_F \) and \( \tau_F \) are
observed fluorescence quantum yield and the average fluorescence lifetime, respectively.
Apparently, in the defunctionalization the fluorescence quantum yield decrease is
correlated with the decrease in \( k_F \) (Table 2.2), consistent with the disappearance of the
extra absorption shoulder, as \( k_F \) and optical absorptivity are correlated and both serve as
measures for electronic transition probabilities.\(^{38}\)
Table 2.2 Fluorescence Decay Results for the Defunctionalization.\(^a\)

<table>
<thead>
<tr>
<th>Sample(^b)</th>
<th>(A_1) (%)</th>
<th>(A_2) (%)</th>
<th>(\tau_{F1}) (ns)</th>
<th>(\tau_{F2}) (ns)</th>
<th>(\chi^2)</th>
<th>(&lt;\tau_F&gt;) (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>8(1)</td>
<td>92(1)</td>
<td>0.8(0.2)</td>
<td>6.9(0.4)</td>
<td></td>
<td>6.8(0.3)</td>
</tr>
<tr>
<td>D(_1)</td>
<td>12(2)</td>
<td>88(2)</td>
<td>0.7(0.2)</td>
<td>4.7(0.4)</td>
<td></td>
<td>4.6(0.4)</td>
</tr>
<tr>
<td>D(_2)</td>
<td>10(2)</td>
<td>90(2)</td>
<td>0.8(0.2)</td>
<td>4.9(0.4)</td>
<td></td>
<td>4.8(0.4)</td>
</tr>
<tr>
<td>D(_3)</td>
<td>9(2)</td>
<td>91(2)</td>
<td>0.7(0.2)</td>
<td>4.8(0.4)</td>
<td></td>
<td>4.7(0.4)</td>
</tr>
<tr>
<td>D(_4)</td>
<td>9(2)</td>
<td>91(2)</td>
<td>0.8(0.2)</td>
<td>5(0.4)</td>
<td></td>
<td>4.9(0.4)</td>
</tr>
</tbody>
</table>

\(^a\) Excitation at 440 nm and the monitored emission centered at 510 nm, with the deconvolution based on bi-exponential fit. \(^b\) Original is the EPA-carbon dots before defunctionalization, and the others are samples with different degrees of defunctionalization collected at different time points in the reaction (D\(_1\): 2 h, D\(_2\): 6 h, D\(_3\): 12 h, and D\(_4\): 20 h and the final defunctionalized sample).
The functionalization and defunctionalization results presented above clearly show the critical role of carbon nanoparticle surface passivation in determining the optical properties of carbon dots. Mechanistically, the fluorescence emissions are attributed to radiative recombinations of photo-generated electrons and holes trapped at diverse surface defect sites. The observed fluorescence properties including quantum yields and decays may be explained in terms of two sequential processes following photoexcitation, the first for the formation of the emissive excited states (quantum yield \( \Phi_1 \)) and then the other from the emissive states for fluorescence (quantum yield \( \Phi_2 \)) and competing nonradiative decay pathways. Thus, the observed fluorescence quantum yields \( (\Phi_F) \) reflect a combination of the two processes, \( \Phi_F = \Phi_1 \Phi_2 \). The obvious decoupling between the observed fluorescence quantum yields and decays in the defunctionalization experiments indicates that the beneficial effect of surface passivation for observed higher fluorescence quantum yields is primarily through \( \Phi_1 \), probably the stabilization of the surface defect sites to facilitate more effective radiative recombinations. Specific to EPA-carbon dots, however, the extra absorption shoulder in the blue (Figure 2.2) as a result of the functionalization may also contribute to a larger \( \Phi_2 \) through enhanced transition probabilities (larger \( k_F \) values). Overall, the results reported here are not only consistent with the presently adopted mechanistic framework for the optical properties of carbon dots, but also providing more details that are valuable to an improved mechanistic understanding. In further investigations, a more systematic evaluation on other similar small amino molecules for functionalization in the preparation of carbon dots will prove interesting and rewarding.
Small carbon nanoparticles were functionalized with EPA molecules for the EPA-carbon dots of bright fluorescence emissions. With EPA of a low molecular weight, these carbon dots are ultra-compact (averaging less than 5 nm in diameter). The solution-phase NMR results support the expected dot structure of carbon nanoparticles surface-attached with EPA species. It should again be emphasized that the amidation reaction used in this study for the functionalization of carbon nanoparticles is specific, with the reaction conditions impossible to cause any "heteroatom doping" in the resulting carbon dots, thus very different from the carbonization reactions or the like used in the synthesis of "graphene quantum dots" without or with surface passivation (the latter being essentially carbon dots of a more graphitic carbon core).\textsuperscript{57-60} As related, the hydrolysis reaction for the defunctionalization is also impossible to reverse any heteroatom doping or structural modifications if there were such doping or modifications in the original functionalization. Thus, none of the observed optical properties in this study could be attributed to effects associated with any doping or structural modifications of the carbon nanoparticles used in the amidation reaction.

On the optical properties, while the EPA-carbon dots share many features found in carbon dots of other surface functionalities, there is an extra absorption shoulder in the blue spectral region into which the excitation results in enhanced green fluorescence emissions. The EPA-carbon dots were defunctionalized by gradually removing the EPA species from the carbon nanoparticles, along with spectroscopic characterizations. The results further confirm the critical role of surface functionalization to the bright
fluorescence emissions of carbon dots. According to systematic measurements of fluorescence quantum yields and decays for the EPA-carbon dots and their gradually defunctionalized samples, two mechanistic conclusions can be made. One is that the enhanced green fluorescence emissions corresponding to the extra blue absorption shoulder are due to significant contributions of larger fluorescence radiative constants, associated with enhanced transition probabilities. The other is that after taking out the special effect discussed in the first conclusion above, the observed fluorescence properties of carbon dots in general can be explained by a combination of two sequential processes following photoexcitation: the formation of the emissive excited states and the decays from these states. Further investigations by using ultrafast spectroscopy techniques are needed.
References


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CHAPTER THREE

PREPARATION AND STUDIES OF CARBON NANOPARTICLES IN
FUNCTIONALIZATION BY ELECTRONIC POLYMERS

3.1 Zero-Dimensional Carbon Allotropes – Carbon Nanoparticles versus Fullerenes in
Functionalization by Electronic Polymers for Different Optical and Redox Properties

3.1.1 Introduction

The discovery and development of nanoscale carbon allotropes began with
fullerenes at the zero-dimension,\textsuperscript{1,2} then one-dimensional carbon nanotubes,\textsuperscript{3-5} and more
recently graphenes in two-dimension.\textsuperscript{6-8} For fullerenes, the widely explored have been
polymer/fullerene nanocomposites,\textsuperscript{9} including polymer-functionalized fullerenes and
those based on fullerene derivatives such as the popular methano-C\textsubscript{60} derivative 1-(3-(methoxycarbonyl)propyl)-1-phenyl[6.6]C\textsubscript{61} (PCBM).\textsuperscript{10} There have been many studies
in which PCBM and similar fullerene derivatives are dispersed in polythiophenes and
related electronic polymer matrices for the resulting materials to serve as critical
components in photovoltaic devices and LEDs.\textsuperscript{11-13} However, the parent C\textsubscript{60}
and derivatives are weak absorbers over most of the visible spectral region and also rather
poor emitters in general, as suggested by results from extensive spectroscopic
investigations.\textsuperscript{14-16} Beyond fullerenes, similarly popular have been the functionalization
and composites of carbon nanotubes and graphenes with the same group of electronic
polymers for a broad range of device and other applications.\textsuperscript{17-19}
Small carbon nanoparticles generally without any defined crystalline structures have recently been exploited for their effective photon-harvesting due to broad optical absorptions that are comparable to those of carbon nanotubes and graphenes,\textsuperscript{20} with the harvested photon energies driving some significant excited state and redox processes relevant to the energy conversion applications.\textsuperscript{20-22} More importantly, with surface passivation of the small carbon nanoparticles, the resulting quantum dot-like entities, commonly referred to as carbon dots (CDots, Figure 3.1),\textsuperscript{23,24} have exhibited optical properties and photoinduced redox characteristics easily rival those of fullerenes.\textsuperscript{25-28} Among the large and ever increasing number of studies on CDots already reported in the literature,\textsuperscript{27-34} most have been on their bright and colorful fluorescence emissions that compete in performance with those of semiconductor quantum dots, including especially their uses as high-performance yet benign and nontoxic fluorescence probes. The photoinduced redox properties and processes in CDots have been explored for their serving as potent photocatalysts,\textsuperscript{21,35} various components in photovoltaic devices and LEDs,\textsuperscript{36-39} and other applications.\textsuperscript{27,28,40} In fact, as suggested by substantial and growing experimental evidence, CDots should be considered as another nanoscale carbon allotrope at the zero-dimension, in addition to fullerenes, representing now a rapidly advancing and expanding research field.\textsuperscript{27-34} For potential optoelectronic applications in particular, critical comparisons between CDots and fullerenes in terms of their functionalization and composites with electronic polymers, and on their associated optical and redox properties will prove highly valuable.
Figure 3.1 A cartoon illustration on CDots, which are generally small carbon nanoparticles with various surface passivation schemes (by attached PVK polymers in this work).
In the reported study, poly(N-vinylcarbazole) (PVK) was selected as representative for its well known hole transport function in photovoltaic devices and LEDs,\textsuperscript{38,41} though the same material configuration and the results and conclusions should be applicable to other electronic polymers. Specifically, PVK-functionalized CDots and C\textsubscript{60}, denoted as PVK-CDots and PVK-C\textsubscript{60}, respectively, were prepared in the copolymerization reaction of N-vinylcarbazole with pre-processed and selected small carbon nanoparticles and C\textsubscript{60}, respectively. The PVK-CDots and PVK-C\textsubscript{60} were compared for their absorption and fluorescence emission properties and photoinduced redox characteristics. The results suggest that CDots could indeed rival fullerenes for applications benefiting from more effective photon-harvesting and excellent electron donor, as well as acceptor, functions.

### 3.1.2 Results and Discussion

A commercially acquired carbon nanopowder sample was processed for the harvesting of smaller carbon nanoparticles, where the processing included refluxing in nitric acid, dialysis, and centrifugation to retain the supernatant as an aqueous dispersion of the targeted nanoparticles.\textsuperscript{42} The nanoparticles (on average around 5 nm in diameter according to transmission electron microscopy imaging results)\textsuperscript{43} were recovered from the dispersion for the copolymerization reaction with N-vinylcarbazole to obtain the PVK-CDots. In the synthesis, N-vinylcarbazole and the small carbon nanoparticles were dispersed in a small amount of DMF to form a homogenous mixture. Upon the removal of solvent, the solid mixture was treated thermally for \textit{in situ} polymerization. The
reaction mixture was dispersed in toluene for purification via repeated precipitation into diethyl ether and washing with diethyl ether. The cleaned sample was dispersed in DMF, followed by centrifugation to collect the supernatant as a solution of the PVK-CDots. The residue was characterized by thermogravimetric analysis (TGA) to determine the content of carbon nanoparticles for mass balance, and the result suggested that a majority of the starting carbon nanoparticles used in the in situ polymerization ended up in the PVK-CDots sample. The optical absorption spectrum of the PVK-CDots in DMF is shown in Figure 3.2. As expected, the visible absorptions in the dots are similar to those of the aqueous dispersed small carbon nanoparticles (Figure 3.2), which were known to be responsible for the photon-harvesting by CDots over the visible spectral region.22
Figure 3.2 Absorption spectra (enlarged for the 600 – 750 nm spectral region in the inset) of the PVK-CDots in DMF solution (——), the PVK-C_{60} in dichlorobenzene solution (- - - -), and also the aqueous suspended small carbon nanoparticles (-.-.-).
As a control, N-vinylcarbazole only without any carbon nanoparticles was thermally treated under the same conditions, yielding PVK polymers similar to those acquired commercially. The PVK polymers are colorless, obviously different from the PVK-CDots in terms of optical absorptions in the visible spectral region (Figure 3.2). However, there was a consideration on the possibility of the PVK-CDots being a simple mixture of PVK polymers and the dispersed small carbon nanoparticles, but such a possibility was quickly eliminated by the observed fluorescence emission properties of the PVK-CDots sample.

Fluorescence spectra of the PVK-CDots in DMF solution are shown in Figure 3.3, which are excitation wavelength dependent in the same pattern as what has been observed in many other CDots of different surface passivation schemes and/or prepared from different synthesis methods.28 Also similar to the other CDots reported in the literature,42,44-46 the PVK-CDots are relatively more fluorescent in the green, with observed quantum yields of more than 30% at 440 nm excitation for emissions around 530 nm (Figure 3.3). The results are comparable with those of CDots in which carbon nanoparticles are chemically functionalized by small amino molecules such as 2,2’-(ethylenedioxy)bis(ethyamine) (EDA, thus EDA-CDots) and 3-ethoxypropylamine (EPA, thus EPA-CDots) under amidation reaction conditions (Figure 3.3).42,45 Fluorescence decays of the PVK-CDots are generally non-exponential, also similar to what have been found for other CDots.26,42,45 The decay curves for 440 nm and 540 nm excitations are rather similar, as shown in Figure 4, and they could be deconvoluted by using a bi-exponential function. The fitting parameters thus obtained (Figure 3.4) were
used to calculate the average fluorescence lifetimes $\langle \tau_F \rangle$ based on the equation $\langle \tau_F \rangle = (A_1\tau_{F1}^2 + A_2\tau_{F2}^2) / (A_1\tau_{F1} + A_2\tau_{F2})$. The $\langle \tau_F \rangle$ values for 440 nm and 540 nm excitations are similar (5.5 ns and 5.7 ns, respectively), as expected, and they are also comparable with those of CDots with surface passivation by small organic molecules.
Figure 3.3 The absorption spectrum of the PVK-CDots in DMF solution (——) is compared with those of the EDA-CDots (-.-.-) and the EPA-CDots (- - - -). Also shown are excitation wavelength dependencies of observed fluorescence quantum yields (○) and fluorescence spectra (in the inset, from left to right corresponding to excitation wavelengths of 400 nm, 440 nm, 480 nm, 520 nm, 560 nm, and 600 nm) of the PVK-CDots in DMF solution.
Figure 3.4 Fluorescence decays and their bi-exponential deconvolution fits for the PVK-CDots in DMF solution at 540 nm (A) and 440 nm (B) excitations are compared with those of the PVK-C60 in dichlorobenzene solution at 540 nm (C) and 700 nm (D) excitations.
The absorption and fluorescence emission properties of the PVK-CDots from the thermally induced copolymerization reaction obviously resemble those of other CDots from different syntheses already reported in the literature, suggesting that the PVK-CDots must be adhering to the general definition on CDots,\textsuperscript{28} with the small carbon nanoparticles surface-passivated by the functionalization of PVK polymers. The FT-IR spectrum of the PVK-CDots is shown in Figure 5, which is apparently dominated by PVK polymers in the dot sample. The spectral features are very close to those of the blank PVK polymers supplied commercially and also from the thermally induced polymerization of \textit{N}-vinylcarbazole in the control experiment discussed above (Figure 3.5). The commercial PVK polymer was specified by the supplier as having an average molecular weight of about 90,000 Da. Thus, the FT-IR results are consistent with the expectation that the thermally induced polymerization reaction yielded PVK polymers. The vinyl stretching mode absorption (around 1,650 cm\textsuperscript{-1}) found in the spectrum of \textit{N}-vinylcarbazole is absent in the spectra of the PVK-CDots and blanks (Figure 3.5), again consistent with the expected polymerization.
Figure 3.5 FT-IR spectra of N-vinylcarbazole (A), PVK-CDots (B), PVK-C60 (C), and PVK polymers from the control experiment (D) and acquired commercially (E).
The outcomes of the copolymerization reaction with the mixture of N-vinylcarbazole and small carbon nanoparticles suggest that the nanoparticles are capable of the same behavior found and established for fullerenes with respect to their similar copolymerization with vinyl monomers. For example, C_60 could be copolymerized with styrene and methyl methacrylate in radical polymerization reactions (thermally initiated or with a radical initiator), yielding copolymers with fullerene cages incorporated in the polymer structure. Here for comparison, C_60 was copolymerized with N-vinylcarbazole in the thermally initiated polymerization under reaction conditions similar to those for styrene-C_60 copolymers reported in the literature. The outcomes on the formation of copolymers in which C_60 cages are incorporated in the PVK polymer structure (denoted as PVK-C_60) were similar to those for copolymers of C_60 with polystyrene and PMMA, including optical properties of the copolymers.

Optical absorption spectrum of the PVK-C_60 in dichlorobenzene solution is also shown in Figure 2. The absorption feature around 708 nm is characteristic of monofunctionalized C_60 cages (commonly referred to as C_60 mono-adducts), suggesting the presence of such structural elements in the copolymer. As also known in the literature, mono- or multi-additions to the C_60 cage would result in significant changes in the optical absorption spectral profile, but hardly any substantial enhancement in absorptivities over most of the visible spectral region. The comparison between absorptivities of the PVK-CDots and PVK-C_60 on the per carbon basis (namely molar carbon atoms in the carbon nanoparticles and C_60 cages in the copolymers) similarly suggests that the former is significantly more effective in the harvesting of visible photons (Figure 3.2).
Parent and derivatized C\textsubscript{60} cages are generally weak in fluorescence emissions\textsuperscript{14,16,57} and ever weaker in multi-adducts including copolymers with incorporated C\textsubscript{60} cages such as those obtained from radical copolymerization of C\textsubscript{60} and styrene\textsuperscript{50,55}. Similarly, the PVK-C\textsubscript{60} was also found to be only weakly emissive, with observed fluorescence quantum yields generally less than 0.1% for excitation wavelengths across the visible spectral region. In fact, because the emissions due to the functionalized C\textsubscript{60} cages are so weak, the observed excitation wavelength dependent fluorescence spectra of the PVK-C\textsubscript{60} (Figure 3.6) are likely contributed significantly by some minor presence of CDots-like species in the sample formed under the thermal processing conditions for the copolymerization reaction. Again in the observed fluorescence spectra (Figure 3.6), emission features around 725 nm characteristic of C\textsubscript{60} mono-adducts\textsuperscript{57} are more distinct, which may be considered as a more reliable signature for the emissive properties of the PVK-C\textsubscript{60}.
Figure 3.6 Absorption (ABS) and fluorescence (FLSC, from left to right corresponding to excitations at 400 nm, 420 nm, 440 nm, 460 nm, 480 nm, 500 nm, 520 nm, and 540 nm) spectra of the PVK-C60 in dichlorobenzene solution.
The optical spectroscopy results presented above clearly demonstrate the major advantages of the PVK-CDots over the PVK-C₆₀ in terms of much more effective photon-harvesting across the visible spectrum and also orders of magnitude brighter fluorescence emissions. In fact, even for excitation of the PVK-C₆₀ at a wavelength like 540 nm, the observed fluorescence emissions are “contaminated” significantly by those due to minor presence of CDots-like species in the sample. Such an assessment is supported by the fluorescence decay results (Figure 3.4) and also by the results of fluorescence quenching associated with photoinduced electron transfers. The average fluorescence lifetime of the PVK-C₆₀ at 700 nm excitation is 2.3 ns (Figure 3.4), close to those of derivatized C₆₀, but much longer at 540 nm excitation, 4.3 ns (Figure 3.4), likely a result of contributions in the observed decays and associated average lifetime calculations by the emission components due to the CDots-like species in the sample.

On the excited state redox characteristics, C₆₀ and derivatives are known as excellent electron acceptors, and CDots are similarly so and also even more potent as electron donors according to already well established literature results. With N,N-diethylaniline (DEA) as electron donor quencher, the Stern-Volmer plots for the PVK-CDots and PVK-C₆₀ (Figure 3.7) are both slightly downward curved at higher DEA concentrations (Figure 3.7). Nevertheless, the more linear portions of the plots (Figure 3.7) correspond to Stern-Volmer constants ($K_{SV}$) of 1.8 M⁻¹ and 5.6 M⁻¹ for the PVK-CDots and PVK-C₆₀, respectively. The former is an order of magnitude smaller than the $K_{SV}$ values found in those CDots with small organic molecules like EDA for surface passivation, and the latter is also substantially smaller than those generally observed for
derivatized C₆₀ molecules (Kᵥ about 17 M⁻¹ for PCBM, as a representative example).⁵⁷
Thus, the PVK functionalization apparently has significant negative effect on the excited
state electron accepting characteristics for both CDots and C₆₀. Also for both of them, the
fluorescence spectral profiles remain unchanged at different DEA concentrations,
suggesting that the emissions being quenched are emission wavelength independent with
respect to the quenching, the same as what have been found in similar fluorescence
quenching studies of other CDots.⁴⁸
Figure 3.7 Stern-Volmer quenching plots for fluorescence intensities of the PVK-CDots in DMF solution (Δ, 440 nm excitation) and the PVK-C$_{60}$ in dichlorobenzene solution (○, 540 nm excitation) at different DEA concentrations.
Figure 3.8 Stern-Volmer quenching plot for fluorescence intensities (440 nm excitation) of the PVK-CDots in DMF solution at different DNT concentrations.
As electron donor in the photoexcited states, the PVK-CDots are as effective as other CDots (the EDA-CDots, for example),\textsuperscript{50} in sharp contrast to the PVK-\textit{C}_{60} of no electron donating ability at all. With the electron deficient molecule 2,4-dinitrotoluene (DNT) as quencher for the fluorescence of the PVK-CDots, the Stern-Volmer plot (Figure 3.8) suggests not only an extremely large $K_{SV}$ value of 65 M$^{-1}$ for the linear portion of the plot, but also static quenching reflected in the upward deviation at still relatively low quencher concentrations. The corresponding quenching rate constant $k_{q}$ ($K_{SV}/\tau F^{0}$) of $1.2 \times 10^{10}$ M$^{-1}$s$^{-1}$ is beyond the upper limit of diffusion control. Such an abnormally efficient redox quenching can be attributed to a large quenching radius beyond the dot surface boundary, similarly responsible for the static quenching (Figure 3.8).\textsuperscript{48,60} On the other hand, like the parent and derivatized \textit{C}_{60} molecules in general, the PVK-\textit{C}_{60} could not serve as electron donor, with the fluorescence emissions around 725 nm remaining unchanged at different DNT concentrations. Since the observed emissions at shorter wavelengths (450 – 680 nm, Figure 3.6) are likely contributed by the CDots-like species in the PVK-\textit{C}_{60} sample, as discussed above, their relatively minor quenching by DNT could be attributed to the same origin.

PVK polymers are known for their hole transport functions in various optoelectronic devices, often based on composites with fullerene derivatives. As presented above, in the composite-like PVK-\textit{C}_{60} sample the PVK could apparently dampen the characteristic electron accepting ability of the fullerene cage, as reflected by the smaller Stern Volmer constant for the fluorescence quenching by DEA (Figure 3.7). The same dampening effect was found in the PVK-CDots, which could also be
considered as PVK/CDots composites, with similarly smaller Stern-Volmer constant for
the electron donor quencher DEA. Mechanistically, the effect is probably not electronic
in nature, as it would have affected the fluorescence properties of the PVK-CDots in the
absence of any external quenchers, inconsistent with their observed high fluorescence
quantum yields (30% or higher). More likely might be “steric effect” due to the PVK
polymers, which prevent the external quencher molecules from getting to the
photoexcited fullerene cages or CDots in the composite-like samples. Interestingly,
however, the dampening effect is apparently absent in the fluorescence quenching of the
PVK-CDots by DNT, which is consistent with the observed substantial static quenching
contributions (Figure 3.8) due to a large effective quenching radius extending beyond the
dot surface.48

As compared in the presentation above, both C60 cages and the small carbon
nanoparticles can participate in radical polymerization reactions with vinyl molecules.
For the PVK-C60, the mode of such participation must be the radical addition to the
fullerene cage, the same as what is known in similarly synthesized styrene-C60 and
PMMA-C60 copolymers.49-56 A similar radical reaction on the carbon nanoparticle surface
is likely in the formation of the PVK-CDots, in which there are probably additional
surface passivation effects provided by the noncovalent interactions with the carbazole
moieties (Figure 3.1). The effectiveness of the carbon nanoparticle surface passivation in
the PVK-CDots is reflected by the high fluorescence performance of the sample, which
serves as an interesting example of potentially far-reaching implications with respect to
alternative surface passivation schemes that adhere to the general definition on CDots but different from what have been studied in various preparations of CDots\textsuperscript{23,28}.

On the PVK-CDots \textit{versus} PVK-C\textsubscript{60} as representatives for CDots \textit{versus} C\textsubscript{60} in electronic polymers represented by PVK and the relevance of these polymeric composite-like materials to optoelectronic and other applications, the results on optical properties and photoinduced redox characteristics show that CDots are more advantageous in terms of significantly more effective photon-harvesting across the visible spectrum and for the harvested photon energies to drive electron accepting and especially highly potent electron donating functions. Small carbon nanoparticles are obviously more abundant and inexpensive in comparison with fullerene C\textsubscript{60}, and their copolymerization with N-vinylcarbazole for the PVK-CDots is efficient with high yields. Therefore, further explorations of CDots copolymerized with PVK and other electronic polymers for uses in various optoelectronic devices and systems should prove interesting and valuable.

\textbf{3.1.3 Conclusion}

Small carbon nanoparticles are similar to fullerene C\textsubscript{60} cages, both zero-dimensional carbon allotropes, on their participation in radical initiated copolymerization reactions with vinyl monomers like N-vinylcarbazole. The resulting PVK-CDots are structurally the carbon nanoparticles with surface passivation by the attached PVK polymers, thus adhering to the general definition on CDots. The PVK-CDots are comparable with and mostly more advantageous than the corresponding PVK-C\textsubscript{60} in terms of optical properties (more specifically photon-harvesting over the visible spectrum
and bright fluorescence emissions) and photoinduced redox characteristics. Since fullerene-based composites with electronic polymers including PVK have found significant applications in optoelectronic devices and systems, similar or more valuable uses of the CDots functionalized by electronic polymers like PVK may be envisaged.

3.1.4 Experimental Section

**Materials.** The carbon nanopowder sample (US1074) was purchased from US Research Nanomaterials, Inc., and C_{60} (purity >98%) from BuckyUSA. N-Vinylcarbazole and poly(N-vinylcarbazole) (PVK, molecular weight ~90,000) were obtained from Acros Organics, N,N-diethylaniline from Avocado, and 2,4-dinitrotoluene from TCI. DMF, o-dichlorobenzene, and toluene were supplied by Burdick & Jackson, diethyl ether by Mallinckrodt Chemicals, and nitric acid by Fisher Scientific.

**Measurement.** UV/vis absorption spectra were recorded on a Shimadzu UV2501-PC spectrophotometer. Fluorescence spectra were acquired on a Jobin-Yvon emission spectrometer equipped with a 450 W xenon source, Gemini-180 excitation and Triax-550 emission monochromators, and a photon counting detector (Hamamatsu R928P PMT at 950 V). The spectra were corrected for nonlinear instrument response by using pre-determined correction factors specific to the emission spectrometer. 9,10-Bis(phenylethynyl)-anthracene in hexane was used as a standard in the determination of fluorescence quantum yields by the relative method (matching the absorbance at the excitation wavelength between the sample and standard solutions and comparing their corresponding integrated total fluorescence intensities). Fluorescence decays were
measured on a Horiba Ultima Extreme spectrometer based on the time-correlated single photon counting (TCSPC) technique, equipped with a SuperK Extreme supercontinuum laser source pulsed at 10 MHz, TDM-800 excitation and TDM-1200 emission monochromators, a R3809-50 MCP-PMT detector operated at 3.0 kV in a thermoelectrically cooled housing, and FluoroHub A+ timing electronics. The time resolution as characterized by the instrumental response function (IRF) of the setup is 100–200 ps (depending on excitation wavelength). Experimental decay curves were fitted with Das6 fluorescence decay analysis software. FT-IR spectra were collected on a Shimadzu IRAffinity-1S spectrometer equipped with the Single Reflection ATR accessory for solid samples. Thermogravimetric analysis (TGA) was carried out on a TA Instruments Q600 system.

**PVK-CDots.** Small carbon nanoparticles were harvested from the commercially acquired carbon nanopowder sample in procedures similar to those reported previously. In a typical experiment, the carbon nanopowders (2 g) were refluxed in concentrated nitric acid (8 M, 200 mL) for 48 h. The reaction mixture was cooled back to ambient temperature, and centrifuged at 1,000 g to discard the supernatant. The residue was re-dispersed in deionized water, dialyzed in a membrane tubing (molecular weight cut-off ~500) against fresh water for 48 h, and then centrifuged at 1,000 g to retain the supernatant as an aqueous dispersion of small carbon nanoparticles.

A solution of \(N\text{-vinylcarbazole}\) in DMF (250 mg/mL, 2 mL) was prepared, and to the solution was added the small carbon nanoparticles (up to 70 mg). The resulting mixture was mildly sonicated, followed by evaporation to remove DMF. The mixture in a
glass tube was thermally treated at 350 °C in a sealed stainless steel reactor for 90 min. Post-treatment, the reaction mixture was collected and dispersed in toluene, and the dispersion was precipitated into diethyl ether and then washed repeatedly with diethyl ether. The brownish solids thus obtained were re-dispersed in DMF, followed by centrifuging at 20,000 g for 30 min to collect the supernatant as the PVK-CDots in a DMF solution.

**PVK-C_{60}**. A C_{60} sample (30 mg) was added to a solution of N-vinylcarbazole in dichlorobenzene (200 mg/mL, 1.5 mL). The resulting mixture was mildly sonicated, followed by evaporation to remove dichlorobenzene. The mixture in a glass tube was thermally treated at 150 °C in a sealed stainless steel reactor for 12 h. Post-treatment, the reaction mixture was collected and dispersed in toluene, and the dispersion was precipitated into hexane, washed repeatedly with hexane, and then re-dispersed in toluene. The dispersion was precipitated into diethyl ether and then washed repeatedly with diethyl ether. The PVK-C_{60} was collected as a reddish brown solid sample soluble in dichlorobenzene.

### 3.2 Supporting Information and Further Discussion

The PVK-CDots in benzene-\textit{d}_6 solution were characterized using $^1$H and $^{13}$C NMR techniques for several reasons. One was to obtain an estimate of the average molecular weight of the PVK polymers in the dot sample based on the established end-group analysis method. As shown in Figure 3.9, the proton signals in the NMR spectrum are too broad to determine if any shifts of the signals are associated with the
end groups. Even though this method could not provide complete results because of this issue, it could still provide some valuable information on the polymers being analyzed. The PVK polymers in the dot sample need to have a number-average molecular weight ($M_n$) larger than 25,000, which is generally considered the limit for end-group analysis using $^1$H NMR.\textsuperscript{62} Further supporting such an assessment is the comparison with the spectrum of commercially acquired PVK polymers with a known $M_n$ value of 90,000 Da (Figure 3.9). The two spectra are rather similar, and they are also similar to the spectrum of blank PVK polymers prepared under the same synthetic conditions without any carbon nanoparticles (Figure 3.9). The latter is not unexpected considering the relatively low number of carbon nanoparticles in the PVK-CDots. The $^{13}$C NMR spectrum of the PVK-CDots is similarly broad and also comparable to those of the blank PVK polymers acquired commercially and prepared under the same conditions but without carbon nanoparticles (Figure 3.10). While the spectral similarities in the $^1$H and $^{13}$C NMR results from the PVK-CDots do not provide any significant structural information, they nevertheless eliminate any detectable presence of other species that might be responsible for the optical properties observed.
Figure 3.9 Proton NMR spectra of PVK-CDots (top), PVK polymers from the control experiment (middle) and those acquired commercially (bottom).
Figure 3.10 Carbon NMR spectra of PVK-CDots (top), PVK polymers from the control experiment (middle) and those acquired commercially (bottom).
The incorporation of the initial carbon nanoparticles into the PVK polymers as a result of the \textit{in situ} polymerization reaction was also made evident in the transmission electron microscopy (TEM) imaging of the PVK-CDots (Figure 3.11). For this imaging, the specimen was prepared by taking a few drops from a diluted solution of the PVK-CDots in toluene and depositing this sample on a silicon oxide-coated copper grid. The small carbon nanoparticles in the dot sample could clearly be identified in the TEM images (Figure 3.11). The relatively low population of the nanoparticles in the specimen is consistent with the generally low particle content in the dot sample (see below). An effort was made to increase the particle counts in the TEM images by depositing the same solution multiple times or by using a more concentrated solution in the preparation of the TEM specimen, but neither resulted in clear TEM images due to interference from the increased number of PVK polymers associated with the low contrast between them and the carbon nanoparticles.
Figure 3.11 TEM imaging of the PVK-CDots.
The synthesized PVK-CDots were found to share solubility with PVK in common organic solvents, allowing for the solution-phase mixing and the subsequent wet-casting of optically transparent PVK films dispersed with fluorescent PVK-CDots (Figure 3.12). A dichlorobenzene/toluene mixture was selected as the solvent because of its intermediate boiling point (thus exhibiting a suitable film-forming rate under ambient conditions). Using a typical fabrication protocol, PVK-CDots were mixed well with neat PVK in solution, followed by evaporating the solvent to obtain the ideal concentration for wet-casting onto a pre-cleaned glass slide. The film appeared homogeneous and optically transparent and became free-standing after a drying period. As the PVK added was transparent and non-emissive in the visible region, the absorption and fluorescence spectra of the PVK/PVK-CDots films were very similar to those from a solution of PVK-CDots. The ease of which PVK-CDots form a film makes their application in photovoltaic devices and LEDs attractive.
Figure 3.12 Absorption (ABS) and fluorescence (FLSC, from left to right corresponding to excitations at 440 nm, 480 nm) spectra for the film of PVK/PVK-CDots (——), with the ABS and FLSC spectra of PVK-CDots in DMF solution (- - - -) also shown for comparison. Inset: photograph for a typical film.
The content of the carbon nanoparticles in the PVK-CDots, which can be varied by altering the reactant mixtures and/or reaction conditions, should be capped at approximately 10 wt% (Figure 3.13), with excess carbon nanoparticles in the initial reactant mixture appearing in the insoluble fraction in the post-reaction workup. Interestingly, similar in situ polymerization reactions incorporating fullerenes in various polymers such as polystyrenes\textsuperscript{50,51} or in reactions to functionalize fullerenes with watersoluble polymers to introduce fullerene cages into aqueous media\textsuperscript{63} also exhibit general caps in terms of the number of fullerene cages in the polymer/fullerene samples that remain soluble in the solvents preferred for the polymers. These caps may represent the limits of the capability of otherwise soluble polymers to “carry” the carbon nanomaterials into the corresponding solvents for solutions or solution-like homogeneous dispersions. According to previous research,\textsuperscript{42} a carbon core of 5 nm in diameter contains approximately 7000 carbon atoms, and based on the carbon content in PVK-CDots from the TGA results, there will be almost 5000 carbazole units for one carbon core. Thus, a network structure of PVK polymers with PVK-CDots as isolate spots embedded in it is proposed here, though the connection between the spots and networks is not understood, the localized spots are apparently very close to or essentially no difference from the well-defined scheme of CDots.\textsuperscript{23}
Figure 3.13 TGA traces (N₂, 10 °C/min) of the PVK-CDots sample (——) and neat PVK polymer (- - - -).
Further investigations are needed for a better understanding of the interaction between core carbon nanoparticle and surface passivation schemes. As suggested by the existence of $C_{60}$ mono-adducts in PVK-$C_{60}$, there might be a similar configuration in PVK-CDots, namely the core carbon nanoparticle copolymerized with PVK polymers. Thus, the structure of PVK-CDots and the interaction between the core carbon nanoparticle and PVK polymers might be different from those of CDots with monomers as surface functionalities, EPA-CDots for example, as it is unlikely that mono-functionalized EPA-CDots would account for the superb optical properties as well as the excellent aqueous dispersibility. In fact, results from a system in which $N$-ethylcarbazole was used to functionalize carbon nanoparticle successfully suggest the interaction of $\pi-\pi$ electrons might account for the connection between the core carbon nanoparticle and carbazole, as there is no vinyl group in $N$-ethylcarbazole. Thus, a similar interaction should be considered in PVK-CDots in addition to copolymerization. Due to the complexity of CDots and the limitation of characterization tools, the structure of CDots remains a mystery, and the current analysis is an oversimplification of the difference between the reality and the optical properties observed and the photoinduced redox characteristics. More research is needed to more fully understand CDots, and as it is conducted, we will improve their performance as a new class of carbon nanomaterials.
References


APPENDICES
Appendix A

Coauthored Publications during My Graduate Study


Carbon Dots’ Antiviral Functions Against Noroviruses

Xiuli Dong1, Marsha M. Moyer1, Fan Yang2, Ya-Ping Sun2 & Liju Yang1

This study reported the first assessment of carbon dots’ (CDots) antiviral activity to human norovirus virus-like-particles (VLPs), GI.1 and GII.4 VLPs. CDots with different surface passivation molecules, 2,2′-(ethylenedioxy)bis(ethyamine) (EDA)-CDots and 3-ethoxypropylamine (EPA)-CDots, were synthesized and evaluated. The results indicated both EDA- and EPA-CDots were highly effective to inhibit both strains of VLPs’ bindings to histo-blood group antigens (HBGAs) receptors on human cells at CDots concentration of 5 µg/mL, with EDA-CDots achieving 100% inhibition and EPA CDots achieving 85–99% inhibition. At low CDots concentration (2 µg/mL), positively charged EDA-CDots exhibited higher inhibitory effect (~82%) than non-charged EPA-CDots (~60%), suggesting the surface charge status of CDots played a role in the interactions between CDots and the negatively charged VLPs. Both types of CDots also exhibited inhibitory effect on VLP’s binding to their respective antibodies, but much less effective than those to HBGA binding. After CDots treatments, VLPs remained intact, and no degradation was observed on VLPs’ capsid proteins. Taken together, the observed antiviral effects of CDots on noroviruses were mainly through the effective inhibition of VLPs’ binding to HBGA receptors and moderate inhibition of VLPs’ binding to their antibodies, without affecting the integrity of viral capsid protein and the viral particle.

Human Norovirus (NoV) is the most common cause of nonbacterial, acute gastroenteritis outbreaks worldwide1, 2, accounting for more than 21 million illnesses and hospitalizations, and at least 570 deaths in the United States each year (Centers for Disease control and Prevention, 2013). NoVs are a group of related non-enveloped, single stranded RNA viruses that have been classified in the Caliciviridae family. NoVs contain six genogroups (from GI to GIV), which can be further divided into different genetic clusters or genotypes based on their capsid sequence1. For example, GI includes nine genotypes and GII contains 22 genotypes1, 3. Genogroups GI, GII, and GIV are responsible for disease in humans4.

NoV is extremely contagious and affects people of all ages. Human NoV transmission occurs by the fecal-oral route, usually through ingestion of contaminated food or water5, by breathing the air near an episode of vomiting, or by direct contact with an infected individual (62–84% of all reported outbreaks). NoV aerosols are formed during vomiting. A single episode of vomiting could release as many as 30 million virus particles6, while fewer than twenty virus particles can cause an infection7. NoV aerosols can also be formed by toilet flushing when vomit or diarrhea is present. The large amount of virus releasing from both fecal material and vomitus of infected individuals and the low infectious dose threshold are the factors that lead to the high number of human NoV annual outbreaks.

Studies have shown that NoVs recognize and interact with human histo-blood group antigens (HBGAs) in intestinal tissues as receptors or attachment factors in a strain-specific manner8, 9. HBGAs are complex carbohydrates and represent terminal structures of glycan chains. They are highly polymorphic and include three major families: the ABO, secretor, and Lewis families. HBGAs are presented abundantly on the surface of mucosal epithelia of gastrointestinal track, where they may function as anchors for NoVs to initiate an infection10. Previous studies suggested that synthetic HBGAs or HBGA-expressing enteric bacteria could enhance NoV infection in B cells11.

The prevention and control of human NoVs infections have been challenging, despite the more significant effort in recent years based on different chemical and physical antiviral methods12–20. Most of these methods have been extensions of their antibacterial uses, whereas NoVs are known to be resistant to commonly used sanitizers and disinfectants21. Among the more recently developed alternative antiviral strategies, the use of nanoparticles

1Biomanufacturing Research Institute and Technology Enterprise (BRITE) and Department of Pharmaceutical Sciences, North Carolina Central University, Durham, NC, 27707, USA. 2Department of Chemistry and Laboratory for Emerging Materials and Technology, Clemson University, Clemson, SC, 29634, USA. Correspondence and requests for materials should be addressed to Y.-P.S. (email: syaping@clemson.edu) or L.Y. (email: lyang@nccu.edu)
has yielded promising results, including for example silver nanoparticles\(^{22}\), gold-copper core-shell nanoparticles\(^{23}\), and TiO\(_2\) nanoparticles coupled with illumination of low-pressure UV light\(^{24}\).

A major difficulty in the study of human NoVs in general has been due to challenges in the cultivation of the virus \(\textit{in vitro}\), despite some progress very recently\(^{25}\), and to a lack of good animal model. Much of the research effort on NoVs has been based on the use of cultivable surrogates such as murine norovirus, feline calicivirus, and poliovirus\(^{16,26}\) and more conveniently the virus-like-particles (VLPs). VLPs are self-assembled VP1 capsid proteins, which are expressed from open read frame 2 (ORF2) as a recombinant protein independent of other viral components. Each VLP is \(\sim 38\) nm in diameter. While the VLPs do not contain the genomic RNA and are replication deficient, their structural and antigenic characteristics are indistinguishable from the native virus\(^{27,28}\). The NoV VLPs have been used as a promising vaccine platform for their ability to elicit a strong humoral and cellular immune response\(^{29}\). The characteristics of NoV VLPs and the easy production systems make them appropriate models for studying NoVs in biological assays and for understanding some specific questions about human NoVs. For example, VLPs were used successfully as a model in our previously reported study on the antiviral activity of gold-copper core-shell nanoparticles\(^{23}\). They have also been used as a model system for studying many other chemical and physical antiviral methods\(^{23}\). They are also useful in modeling virus-cell interactions\(^{27,28}\), and in identifying NoV binding receptors on human cells such as HBGAs\(^{30}\). In the work reported here, we used NoV VLPs as a model of human NoVs to explore the potential antiviral functions of the recently developed carbon dots.

Carbon dots (CDots)\(^{31}\) are small carbon nanoparticles with surface passivation, for which more effective has been the chemical functionalization of organic molecules\(^{32,33}\). As a new class of quantum dot-like nanomaterials, CDots possess properties of bright fluorescence, no toxicity \(\textit{in vitro} \) and \(\textit{in vivo}\) environmentally benign, simple synthetic routes, as well as photocatalytic functions resembling those found in conventional nanoscale semiconductors\(^{34-36}\). Many potential applications of CDots are being pursued across many fields such as chemical and biological sensing, bioimaging, nanomedicine, photocatalysis, and electrocatalysis\(^{34}\). Among the unique properties of CDots that are more relevant to the study reported here is the photo-activated antimicrobial function\(^{36,37}\). In fact, CDots with visible light illumination were highly effective in the inhibition of \(E. \text{coli}\) cell activities in several experimental settings, which has been attributed mechanistically to the photodynamic effect in CDots. Interestingly and surprisingly, we found in this study the significant antiviral activity of CDots toward NoV VLPs. More specifically, effects of the CDots on VLPs’ HBGA binding, antibody binding, and on the integrity of capsid protein and integrity of VLPs particles were examined. Mechanistic implications of the results are discussed.

### Materials and Methods

#### Human NoV VLPs and antibodies.

Human NoV GI.1 VLPs and GII.4 VLPs, and their respective monoclonal antibodies (anti-GI.1 VLP antibody mAb3901 and anti-GII.4 VLP antibody NS14), were generously provided by Dr. Robert Atmar’s laboratory at Baylor College of Medicine (Houston, TX). The secondary antibody used in ELISA tests was goat anti-mouse IgG H&L antibody conjugated to horseradish peroxidase (HRP), which was purchased from Abcam (Cambridge, MA). The secondary antibody used in western blot assays was goat anti-mouse antibody labeled with IRDye\(^{88}\) 800CW, which was purchased from LI-COR Biosciences (Lincoln, NE).

#### CDots.

The CDots were synthesized by chemical functionalization of small carbon nanoparticles, which were harvested from the commercially acquired carbon nano-powders (US Research Nanomaterials, Inc.) in procedures similar to those reported previously\(^{37,38}\). In a typical experiment, a sample of carbon nano-powders (2 g) was refluxed in aqueous nitric acid (8 M, 200 mL) for 48 h. The reaction mixture was cooled back to room temperature, and centrifuged at 1,000 \(g\) to discard the supernatant. The residue was re-dispersed in deionized water, dialyzed in a membrane tubing (molecular weight cut-off \(\sim 500\)) against fresh water for 48 h, and then centrifuged at 1,000 \(g\) to retain the supernatant. Upon the removal of water, small carbon nanoparticles were recovered and used in the functionalization reaction with 2,2′-(ethylenedioxy)bis(ethylenimine) (EDA, Sigma-Aldrich)\(^{39}\) or 3-ethoxypropylamine (EPA, TCI America)\(^{38,39}\) to yield EDA-CDots or EPA-CDots, respectively (Fig. 1).

For the synthesis of EDA-CDots\(^{37,38}\), the small carbon nanoparticles were refluxed in neat thiouyl chloride for 12 h. Upon the removal of excess thiouyl chloride, the treated sample (50 mg) was mixed well with carefully dried EDA liquid in a round-bottom flask, heated to 120 °C, and vigorously stirred under nitrogen protection for 3 days. The reaction mixture back at room temperature was dispersed in water and then centrifuged at 20,000 \(g\) to retain the supernatant. It was dialyzed at 20,000 \(g\) temperature, and centrifuged at 20,000 \(g\) for 5 min. The supernatant was collected and diluted to 1:2000 in PBS. For coating the plates with HBGAs, aliquot of 50 µL saliva dilution was used to coat 96-well plates at 4 °C overnight. Unbound saliva was removed and the wells were rinsed three times with PBS. The plates were then blocked with 100 µL Super-Block T20 (PBS) Blocking Buffer (Thermo Scientific Inc., Waltham, MA) for 1 h and rinsed with PBS twice.

Saliva-based histo-blood group antigen (HBGA) receptor binding assay to evaluate the effect of CDots treatment on VLPs’ HBGA binding capacity.

The effect of CDots treatment on VLPs was evaluated using a saliva-based HBGA binding assay according to a protocol reported previously with minor modifications\(^{48,49}\). Briefly, saliva samples from healthy adult volunteers, including blood type A, B, and O, were collected. Saliva samples were immediately boiled for 5 min and centrifuged at 10,000 \(g\) for 5 min. The supernatant was collected and diluted to 1:2000 in PBS. For coating the plates with HBGAs, aliquot of 50 µL saliva dilution was used to coat 96-well plates at 4°C overnight. Unbound saliva was removed and the wells were rinsed three times with PBS. The plates were then blocked with 100 µL Super-Block T20 (PBS) Blocking Buffer (Thermo Scientific Inc., Waltham, MA) for 1 h and rinsed with PBS twice.
For the HBGA binding assay, aliquots of 50 µL of 1.5 µg/mL VLPs, which were pretreated with 2 or 5 µg/mL EDA- or EPA- CDots at room temperature for 15 min, were added into the wells and incubated 1 h at room temperature. The unbound VLPs were removed and the wells were rinsed with PBST twice. The GI.1 or GII.4 VLPs binding to HBGAs in the wells were detected by using 1 µg/mL primary antibody mAb 3901 (to GI.1) or mAb NS14 (to GII.4), respectively, followed by the addition of 0.5 µg/mL secondary antibody–HRP-labeled goat anti-mouse IgG antibody. The antibody binding conditions included 1 h incubation at 37 °C and rinse with PBST twice. The final products were developed by adding 3,3′,5,5′-tetramethylbenzidine (TMB) peroxidase substrate (KPL, Gaithersburg, MD), and the absorbance reading at the wavelength of 450 nm was performed using a Spectra-Max M5 plate reader (Molecular Devices, Sunnyvale, CA).

**ELISA test to evaluate the effect of CDots treatments on human NoV VLPs’ antibody binding capacity.** EDA- and EPA- CDots with various concentrations ranging from 0 to 60 µg/mL were used to treat 1 µg/mL GI.1 or GII.4 VLPs in medium-binding 96-well polystyrene plates (Costar #3591; Corning Incorporated, Corning, NY). 1× PBS was added to reach the final volume of 50 µL in each reaction. The plates were constantly agitated on the shaker at the setting level of 2 at room temperature for 30 min, followed by 30 min incubation without agitation. The reaction solutions were discarded and the wells were washed with 100 µL 1× PBS twice. The wells were then blocked with 100 µL Super-Block T20 (PBS) Blocking Buffer (Thermo Scientific Inc., Waltham, MA) for 1 h. After the blocking solution was discarded, each well was washed with 100 µL PBST twice. Then, aliquots of 50 µL of 1 µg/mL anti-GI.1 VLP antibody mAb 3901 or anti-GII.4 VLP antibody mAb NS14 were added to each well to bind with the bound GI.1 or GII.4 VLPs, respectively, followed by 1 h incubation at 37 °C. After wash with PBST twice, aliquot of 50 µL of 1 µg/mL HRP-labeled goat anti-mouse IgG antibody solution was added to each well, and the plates were incubated at 37 °C for 1 h. After the incubation, the plates were washed with PBST, and the final products were developed using the TMB kits, and the absorbance at 450 nm in each well was measured.

**SDS-PAGE and western blotting for evaluation of the effect of CDots on VLPs capsid protein.** EDA- and EPA- CDots at the concentration of 20 or 60 µg/mL were used to treat 33.3 µg/mL GI.1 or GII.4 VLPs in 1.5 mL centrifuge tubes. 1× PBS was added to reach the final volume of 50 µL in each reaction. The tubes were constantly agitated on a shaker (Lab-Line instruments Inc., Melrose Park, IL) at the setting level of 2 at the room temperature for 30 min. After the CDots treatments, each tube was added with 5 µL of 1× NuPAGE® LDS Sample buffer (Thermo Fisher Scientific, Waltham, MA), 2 µL of 1 M DTT, and 3 µL deionized water (DI-H2O). All the samples were incubated at 70–80 °C for 10 min and then loaded on 2 of precast 1.0 mm × 10-well NuPAGE® 4–12% Bis-Tris gels (Life Technologies, Grand Island, NY). The loading volume was 10 µL for each well. The gels were run in 1× MOPS SDS running buffer (Invitrogen, Carlsbad, CA) at 200 V for 1 h. One gel was used for staining, while the other was for western blotting. The gel for staining was prefixed with a 50% methanol and 7% acetic acid solution for 15 min and then washed with DI-H2O for 5 min, three times. The GelCode Blue stain (Pierce Biotechnologies, Rockford, IL) was used to stain the gel with constantly shaking for 1 h, followed by 1 h de-staining step in DI-H2O. The gel was then imaged using a LI-COR Odyssey Infra-red Imaging System (LI-COR Biotechnology, Lincoln, NE).

For western blotting, the gel was transferred to an Odyssey® nitrocellulose membrane (LI-COR Biotechnology, Lincoln, NE) using 1× NuPAGE® Transfer Buffer plus 10% MeOH and Hoefer Semi-Dry Transfer Apparatus (Hoefer Inc., San Francisco, CA) at 25 V for 1 h. The membrane was then blocked with 10 mL of 1:1 blocking buffer (Rockland Immuno-chemicals Inc., Limerick, PA) and PBS at room temperature for 1 h. The primary antibody treatment was performed by soaking the membrane in 10 mL of 1:1 PBST and blocking buffer, to which...
4.11 µg of mouse monoclonal anti-GI.1 VLP antibody mAb 3901 or anti-GII.4 VLP antibody mAb NS14 had been added, followed by incubation at 4 °C overnight with gently shaking. Then, the antibody solution was discarded, and the membrane was washed 5 times, each with PBS plus 0.05% Tween 20 (PBST) for 5 min, and then treated with 0.5 µg of goat anti-mouse IRDye® 800CW antibodies in 10 mL of 1:1 PBST and blocking buffer at room temperature for 1 h. After washed 5 × 5 min with PBST under shaking, the membrane was soaked in DI-H₂O and then imaged using the LI-COR Odyssey Infra-red Imaging System.

Transmission electron microscopic (TEM) imaging. GI.1 and GII.4 VLP samples were treated with or without CDots, then 10 µL of each sample was placed on a formvar/carbon TEM grid (Electron Microscopy Sciences, Hatfield, PA) for 30 min. All grids were gently wicked to remove the fluid on the surfaces by the use of filter paper. The grids were stained with 2% uranyl acetate for 60 s and TEM images were acquired using a FEI Technai G2 Twin TEM (Hillsboro, OR) in the Shared Materials Instrumentation Facility (SMIF) at Duke University.

Results and Discussion
Inhibitory effect of CDots on VLPs' binding to HBGA receptors.
NoVs recognize human HBGAs as receptors or attachment factors, such binding events play an important role in host susceptibility to NoV infection. The binding of norovirus to HBGAs has been found to be highly diverse but strain-specific. Several binding patterns have been identified and grouped into two major binding groups based on the binding of 14 norovirus strains to HBGAs, and a model of norovirus/HBGA binding has been proposed. A retrospective study showed that type O individuals had a significant higher infection rate than those with other blood types, while other studies showed Norwalk VLPs lacked the binding to saliva samples collected from nonsecretors, and saliva from type B individuals did not bind or weakly bound to Norwalk virus. Therefore, in this study, we examined the effect of CDots on GI.1 and GII.4 VLPs' binding to saliva HBGAs from blood Type A, B, and O.

Figure 2 shows the inhibition percentages of VLPs' binding to type A, B, O saliva HBGA receptors by the treatments with EDA- and EPA-CDots, calculated using the untreated samples as the controls (100% binding). Figure 2A shows the inhibition effects of the treatment with the two types of CDots to the two strains of VLPs on their binding to type A HBGA receptors. For GI.1 VLPs treated with EDA-CDots at 5 µg/mL, the bindings to type A saliva HBGA receptors were completely inhibited (100% inhibition, Fig. 2A), indicating highly efficient inhibition effect of EDA-CDots on GI.1 VLP's binding to HBGA receptors. The same quantitative inhibition (100%) was observed in GII.4 VLP bindings to the type A HBGA receptors with the treatment of 5 µg/mL EDA-CDots (Fig. 2A). The inhibitory effect remained strong even at lower CDot concentrations, such as the more than 80% inhibition in...
Inhibitory effects of CDots on VLPs’ binding to their antibodies. We further examined the effect of CDots treatment on VLPs’ binding to their antibodies. Figure 3A shows the inhibition percentages of EDA-CDots treatment to both GI.1 and GII.4 VLP’s binding activities to their antibodies (mAb 3901 for GI.1 and mAb NS14 to GII.4) at dot concentrations of 0 to 32 µg/mL. For 2 µg/mL EDA-CDots as an example, GI.1 and GII.4 VLP’s binding activities to their respective antibodies were inhibited by ~54% and ~32%, respectively. At a higher EDA-CDots concentration of 8 µg/mL, the inhibition percentages improved to 90% and 87% for GI.1 and GII.4 VLPs, respectively. The results indicated that the two strains of VLPs, EDA-CDots are somewhat more effective in the inhibition of GI.1 VLPs’ binding to type A HBGA receptors than the inhibition of GII.4 VLPs’ binding to type B HBGA receptors. The dot concentration dependence of the interactions of the CDots with VLPs and the associated inhibition effects are likely very complex. On possible mechanisms for the observed strong inhibitory effects of CDots, one is such that the CDots would bind to the surface of VLPs and physically block the active sites on the VLPs used for bindings to the HBGA receptors. Based on the X-ray crystal structure on the prototype GI.1 of NoV, it contains two domains: the shell (S) and the protruding (P) domain, and the HBGA receptor binding interfaces are located at the top of the P domain, containing carbohydrate binding pockets. These pockets involve several scattered amino acid residues that form extensive hydrogen bond network with individual saccharides, thus stabilizing the binding of HBGAs to the virus capsid protein. Although the binding of norovirus with human HBGA is a typical protein-carbohydrate interaction in which the protruding domain of the viral capsid protein serves an interface for the oligosaccharide side-chains of the HBGAs, some of the complexities in the HBGA binding interactions have been discussed in the literature, including capsid P domain loop movements, alternative HBGA conformations, and HBGA rotations. In fact, the blocking of NoV HBGA binding sites has been used as a surrogate for a NoV neutralization assay by using sera from immunized animals or infected humans. It was found that the ability of sera to block VLP-HBGA interactions could be correlated with the protection against infection in NoV-vaccinated chimpanzees and against the illness among infected human volunteers. According to these reported studies, the blocking of the HuNoV capsid from recognizing their binding sites on host cells represents a promising strategy in preventing HuNoV infection. Thus, the observed effective inhibition of the NoV VLPs by the CDots (Fig. 2) may be considered as an application of such a strategy.

Interactions between various carbon nanomaterials and proteins in different mechanisms have been well-documented in the literature, as relevant to the expected interactions of CDots with VLPs capsid proteins. For example, it is known that carbon nanotubes (CNTs) can nonspecifically bind to proteins through complementary charges, π-π stacking, and/or hydrophobic interactions. Analyses of the binding between C60 (a special type of carbon nanoparticles) and lysozyme revealed that the primary driving force for the binding is van der Waals interaction, while polar solvation and entropy are detrimental to the binding. More relevant to the blocking of receptor sites, it was demonstrated that C60 could inhibit the activity of HIV-protasees by integrating with proteins to form hybrid functional assemblies. Therefore, a conceptually analogous explanation on the observed inhibition of NoV VLPs by the CDots (Fig. 2) may be considered as an application of such a strategy.

EDA-CDots were somewhat less effective in the inhibitory effect on both GI.1 and GII.4 VLPs’ binding to their respective antibodies, as compared in Fig. 3B. The treatment with 8 µg/mL of EPA-CDots only resulted in 1% and 7.3% of inhibition to GI.1 and GII.4 VLPs’ binding to their antibodies, much less than those achieved with EDA-CDots (Fig. 3A). Even with the high EPA-CDots concentration of 32 µg/mL, the inhibition percentages for the binding of GI.1 and GII.4 VLPs to their antibodies were only ~27% and ~10%, respectively. However, a further increase in the EPA-CDots concentration to 64 µg/mL, the inhibition percentages for the binding of GI.1 and GII.4 VLPs to their antibodies improved to ~33% and ~26%, respectively, showing no saturation effect. The obviously less effective inhibition by EPA-CDots than that by EDA-CDots may again be attributed to the different surface charge status between the two types of CDots, as similarly discussed above on the inhibition of the VLPs’ binding to HBGA receptors.
In both EDA- and EPA-CDots treatments, GI.1 VLPs were more effectively inhibited in their binding to mAb3901 antibodies than GII.4 VLPs’ bindings to mAb NS14 across the different CDots concentrations (Fig. 3A and B). This might be due to the capsid structure difference in the two strains of VLPs involving VLP-antibody interactions. Interestingly, however, no significant difference was observed in the CDots’ inhibitory effect on GI.1 and GII.4 VLPs’ bindings to HBGA receptors (Fig. 2). Nevertheless, in the literature the difference in capsid structure in NoV GI.1 and GII.4 was found to be a factor accounting for variations in some other antiviral methods. For example, GI.1 VLPs were found to be more vulnerable to high-pressure processing (HPP) than that of the GII.4 strain55, where the disruption of viral envelope and/or capsid structure, not the degradation of the viral protein or genome, was the primary mechanism of HPP55.

The treatment with CDots was clearly more effective in inhibiting VLPs’ binding to HBGA than to their antibodies. In a comparison of the results in Figs 2 and 3, the VLPs’ binding to HBGA was quantitatively 100% inhibited by 5µg/mL EDA-CDots for both GI.1 and GII.4 VLPs versus even at a higher EDA-CDots concentration of

Figure 3. Inhibition effects of EDA-CDots and EPA-CDots on GI.1 and GII.4 VLPs’ binding to their respective antibodies. (A) EDA-CDots and (B) EPA-CDots. Statistic analysis was performed using SAS 9.2. Different letters on the columns indicate significant differences at P < 0.05, while the same letters indicate no significant difference.

Figure 4. The results of SDS-PAGE gel (top) and Western blot (bottom) analysis of GI.1 VLPs (A) and GII.4 VLPs (B) after CDots treatments. Lane 1: VLPs control; Lane 2, 3: VLPs treated with 20µg/mL of EDA-CDots and EPA-CDots, respectively; Lane 4, 5: VLPs treated with 60µg/mL of EDA-CDots, and EPA-CDots, respectively.
8 μg/mL for only ~70–90% inhibition of VLP’s binding to their respective antibodies. The difference in inhibition effectiveness was more substantial in the use of EPA-CDots, as 5 μg/mL of the dots could inhibit ~85–90% HBGA binding versus 64 μg/mL of the dots only inhibiting ~30% of VLPs’ binding to their antibodies. Again, the results in this study and others (X. F. Zhang et al., 2013) suggested that the CDots treatment may be a potential strategy to prevent the initial access or spread of NoV to humans by effectively inhibiting NoV binding to HBGA receptors.

No degradation of VLPs’ capsid protein by CDots treatments. To gain some mechanistic insights into the inhibitory effect of CDots on VLPs’ binding capacity to their antibodies and HBGA receptors, we further examined the possibility of VLPs’ capsid proteins being degraded by CDots treatments. Briefly, GI.1 and GII.4 VLPs were treated with EDA- and EPA-CDots at two different doses (20 μg/mL and 60 μg/mL). The treated and untreated VLPs were analyzed by SDS-PAGE gel, followed by GelCode Blue staining. As shown in Fig. 4 (top), both GI.1 and GII.4 VLPs showed clear bands at ~50 KDa, likely the shifted bands of the full length capsid protein (VP1, ~58KDa), and the other bands at smaller sizes were most likely protein fragments from the capsid protein. Importantly, the protein band patterns and their abundance did not change after CDots treatments, even for the samples treated with 60 μg/mL CDots exhibiting no significant difference from untreated control samples, suggesting that VLPs’ capsid proteins were not degraded by CDots treatments, and VLP capsid protein structures remained intact.

We also examined the possibility of the proteins being antigenically changed after CDots treatments by western blotting using mAb 3901 against GI.1 VLPs and mAb NS14 against GII.4 VLPs. As shown in Fig. 4 (bottom), the abundance of VP1 and other protein fragments detected by western blotting in GI.1 and GII.4 VLPs did not change after CDots treatments. For the protein bands in GI.1 VLPs as an example, it is known that mAb 3901 can bind to either the full-length (58 KDa) capsid protein or a 32 KDa protein fragment in the P domain, recognizing a continuous epitope on the C-terminal of the capsid protein. The antibody mAb 3901 also recognizes a domain between amino acid 453 and amino acid 495, and the lower band in the western blot is likely a fragment that contains this sequence. Similarly for GII.4 VLPs, the mAb NS 14 binds to the capsid protein and other protein fragments that contain the recognized epitopes. Clearly, for both GI.1 and GII.4 VLPs, the protein band patterns detected in western blotting were essentially the same as those observed in SDS-PAGE detected by GelCode Blue staining. Therefore, the results demonstrated that CDots treatments did not degrade the viral protein, as the viral proteins still retained correct primary amino acid sequences and were able to react with their antibodies.

No damage on the integrity of VLP particles by CDots treatments. Shown in Fig. 5 are images of untreated GI.1 and GII.4 VLPs and the VLPs treated with EDA- and EPA-CDots. These images indicated that...
there were no changes in GI.1 and GII.4 VLP morphologies after CDots treatments, nor server aggregation of the VLPs. The observation might be expected for the fact that CDots have been studied extensively as fluorescence stain for cell imaging, causing no cell morphology changes. CDots are also known for their low to no cytotoxicity and high biocompatibility. For example, normal growths of zebrafish larvae were observed after being soaked in 1.5 mg/mL CDots solution. HeLa cell viability was over 90% after being incubated with 500 µg/mL of CDots for 24 h. The observation that the morphology and integrity of VLPs remained unchanged after CDots treatments is consistent with these and other results reported in the literature.

Conclusions
This study explored the effects of EDA-CDots and EPA-CDots on NoVs GI.1 and GII.4 VLPs. The results demonstrated that the treatment with CDots effectively inhibited VLPs’ binding to saliva HBGA receptors (all three types A, B, and O), without degrading VLP capsid proteins or affecting the integrity and morphology of the VLP particles. Between the two types of CDots, the positively charged EDA-CDots were much more effective than the non-charged EPA-CDots in inhibiting the binding of VLPs to HBGA receptors, due to more favorable binding between EDA-CDots and negatively charged VLPs. These CDots also showed inhibitory effect on VLPs’ binding to their respective antibodies, but much less effective compared to those inhibitions to HBGA receptors. Nevertheless, the results from this study showed the proof-of-concept on CDots’ antiviral function through the inhibition of virus binding to HBGA receptors, which could be a promising strategy in preventing HuNoV infection/spread by disabling HuNoV recognizing their binding sites on host cells. As there is no effective vaccine for NoVs, effective hand washing and cleaning of contaminated sites are recommended practices for prevention of NoV infection/spread, examples of potential applications include CDots-containing antiviral sprays for sanitizing NoVs contaminated sites, such as surfaces or instruments in hospital, patient vomiting on carpet/floor, bathroom toilet after patient diarrhea. CDots can also be incorporated into routine hand wash soaps for antiviral purpose. For cases involving aerosol NoVs, incorporating CDots agents into air filtration devices may be explored. Though the practical application for such purpose is likely more complex and requires additional investigations, the reported initial assessment of CDots’ antiviral function is highly valuable, and will be significantly contributing to the knowledge matrix for the broad implications and application potential of CDots.

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Author Contributions

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Photoexcited state properties of carbon dots from thermally induced functionalization of carbon nanoparticles

Yin Hu, a Mohamad M. Al Awak, b Fan Yang, a Sijia Yan, a Qingwu Xiong, a Ping Wang, a Yongan Tang, c Liju Yang,* b Gregory E. LeCroy, a Xiaofang Hou, a Ping Wang, a Yongan Tang, c Liju Yang,* b Gregory E. LeCroy, a Xiaofang Hou, a Christopher E. Bunker, d Linxi Xu, a Nicholas Tomlinson a and Ya-Ping Sun* a

Carbon dots are small carbon nanoparticles with various surface passivation schemes, in which more effective has been the deliberate chemical functionalization of the nanoparticles for brighter fluorescence emissions, even though the synthesis method is more tedious and subject to some limitations in the selection of functionalization molecules. Another more popular synthesis method has been the carbonization of organic species, with the method being more efficient and versatile, but less controllable regarding synthesis and achieving the desired dot structure and performance. In this work, a hybrid approach combining the advantageous characteristics of the two synthesis methods was applied to the preparation of carbon dots using polyethyleneimine (PEI) for surface passivation, where pre-processed and selected small carbon nanoparticles were functionalized with PEI by microwave-induced thermal reactions. Their optical absorption and fluorescence emission properties were evaluated, and the results suggested that the carbon dots thus prepared shared the same photoexcited state characteristics with those from the deliberate chemical functionalization, including comparable fluorescence colors and other properties. A further demonstration of the similarity in photoexcited state properties was based on the same visible light-activated bactericidal functions of the PEI-carbon dots as those found in carbon dots from the deliberate chemical functionalization method. The advantages and potential limitations of the hybrid approach for more controllable yet versatile and efficient syntheses of carbon dots are highlighted and discussed.

Introduction

Carbon “quantum” dots or more appropriately named carbon dots (for the lack of classical quantum confinement in these fluorescent carbon nanomaterials)1–3 have attracted much recent attention,2–9 from simple curiosity or fascination on the fact that any “carbon dirt” could be made to exhibit colorful fluorescence emissions8–15 to the exploration of their various potential technological applications.2–4,8,16–20 In fact, with a quick search of recent literature one would conclude that research on carbon dots has emerged as a highly active and rapidly expanding field, whose broad impacts similar to or even beyond those already derived from conventional semiconductor quantum dots (QDs)21–26 may be envisaged.

Carbon dots are generally small carbon nanoparticles with various surface passivation schemes (Fig. 1),1–3 in which more effective has been the deliberate chemical functionalization of the nanoparticles by organic molecules or biological species for more intense fluorescence emissions in the visible spectrum, extending to the near-IR.2–4,27 Among the more popular approaches for the synthesis of carbon dots have been the deliberate chemical functionalization of small carbon nanoparticles1,27–29 and carbonization (often in “one-pot”) of organic or other carbon-containing precursors.2,8,30,31 The deliberate functionalization approach has been successful in terms of producing structurally well-defined carbon dots of high fluorescence quantum yields (more than 50% in some configurations),27 but the synthesis is more tedious and subject to some limitations in the selection of molecules for functionalization. The carbonization approach is more efficient and versatile, compatible with a diverse selection...
of precursors and functionalization molecules or species, but less controllable both in the synthesis and for the desired structures of produced carbon dots, among other processing and performance issues.\(^2\) Thus, an interesting and useful strategy is to combine the advantageous characteristics of the two synthetic approaches for more controllable yet efficient and versatile preparations of carbon dots. Specifically for such a hybrid approach,\(^3\) the pre-processed and selected small carbon nanoparticles are used as a precursor, but instead of chemical functionalization reactions, the molecules or species designed for surface passivation are “attached to” (or more like “welded onto”) the carbon nanoparticles in thermally induced reactions, in which the molecules for passivation may also be slightly or partially carbonized in the reactions (Fig. 1). Nevertheless, the hybrid approach still adheres closely to the definition of carbon dots as surface-passivated small carbon nanoparticles.

In this work, the hybrid approach was applied to the preparation of carbon dots using polyethyleneimine (PEI) for surface passivation (Fig. 1), where small carbon nanoparticles from the processing of a commercially supplied carbon nanopowder sample were functionalized with PEI in microwave-induced thermal reactions. The optical absorption and fluorescence emission properties were evaluated, and the results suggested that the carbon dots thus prepared shared the same photoexcited state characteristics with those synthesized by the deliberate chemical functionalization approach (such as the carbon dots with EDA for surface functionalization, Fig. 1), including comparable fluorescence colors and other properties. A further demonstration on the similarity in photoexcited state properties was based on the same visible light-activated bactericidal functions of the PEI-carbon dots as those found in carbon dots synthesized by the deliberate chemical functionalization approach. The advantages and potential limitations of the hybrid approach for more controllable yet versatile and efficient syntheses of carbon dots with desired photoexcited state properties are highlighted and discussed.

Results and discussion

A commercially acquired carbon nanopowder sample was used as a precursor in the processing employed to harvest small carbon nanoparticles. Briefly, the as-supplied carbon nanopowder sample was refluxed in nitric acid, followed by dialysis and centrifugation to obtain mostly smaller carbon nanoparticles in an aqueous suspension,\(^2\) which appeared transparent and solution-like. The observed absorption spectrum of the suspension was similar to those of similarly processed carbon nanoparticles reported previously, so were the relatively weak fluorescence emission spectra.\(^3\) According to atomic force microscopy (AFM) results, these particles were on the order of 5 nm in diameter, also similar to those reported previously.

An oligomeric polyethyleneimine (PEI) of a more branched structure, which contains a significant number of primary amine moieties and is structurally more compact, was used for the functionalization of the carbon nanoparticles. Experimentally, carbon nanoparticles were mixed with PEI and a small amount of ethanol via vigorous sonication at a temperature slightly above the ambient temperature, followed by the removal of ethanol via evaporation. The resulting mixture was heated in a conventional microwave oven following a multiple-cycle regimen such that the sample was heated until smoke started to appear, then cooled in the ambient temperature for a short period of time, and the same heating and cooling processes were repeated for a total of up to 30 heating–cooling cycles. Post-processing, the sample again at ambient temperature was dispersed in water with vigorous sonication. The resulting aqueous dispersion was centrifuged at 20,000g to collect the supernatant, followed by dialysis in a membrane tubing against fresh water to remove unreacted PEI and other small molecular impurities to obtain the PEI-carbon dots as a clear solution (Fig. 2). The results of AFM analyses of the PEI-carbon dot sample suggest that these dots are size-wise relatively narrowly distributed, on average are 6.5 nm in diameter with the size distribution standard deviation a little more than 1 nm (Fig. 3).

The absorption spectrum of the PEI-carbon dots in aqueous solution is shown in Fig. 2, which is similar to those of the precursor carbon nanoparticles and also carbon dots prepared from deliberate chemical functionalization (such as EDA-carbon dots, Fig. 2),\(^2\) supporting the notion that the optical absorption in carbon dots is due to electronic transitions in the core carbon nanoparticles.\(^3\) Also shown in Fig. 2 is the fluorescence spectrum of the PEI-carbon dots in an aqueous solution at 400 nm excitation, which is again comparable with that of the EDA-carbon dots.\(^2\)

The PEI-carbon dots in solution were characterized by using a solution-phase NMR technique, and the results were compared with those of free PEI used in the functionalization reaction.
As shown in Fig. 4, the proton NMR signals of the PEI-carbon dots in deuterated water are significantly broader than those of free PEI, consistent with the expected lower mobility of the PEI species attached to carbon nanoparticles. A similar broadening effect was observed in carbon dots prepared by deliberate chemical functionalization with small organic molecules, such as in the EDA-carbon dots, though to a somewhat lesser extent, because signals of the free PEI are already broad (Fig. 4). Overall, the proton NMR signals of the PEI-carbon dots in reference to those of free PEI could be assigned to two groups, one for the $\alpha$ protons, which is downfield-shifted from that of free PEI (Fig. 4), and might be attributed to some de-shielding effect that resulted from the binding and/or strong interactions of the amino groups with carbon nanoparticles, and the other for the $\beta$ and $\gamma$ protons (Fig. 4). The relative integrations between the $\alpha$ and $\beta + \gamma$ proton signals (1-to-1.1) are unchanged from free PEI to the PEI-carbon dots, suggesting no major structural changes in the particle-bound PEI species.

Fluorescence spectra of the PEI-carbon dots in aqueous solution were also measured more systematically as a function of excitation wavelengths. Similar to those found in carbon dots from other syntheses (again the EDA-carbon dots, for example), the excitation wavelength dependence exhibited progressive red shifts and a narrowing of the emission band width with excitation at longer wavelengths (Fig. 5). The excitation wavelength dependence of fluorescence quantum yields followed a similar pattern, as also shown in Fig. 5, again similar to those of carbon dots obtained from other syntheses. The dependencies of fluorescence spectra and quantum yields on excitation wavelengths have been rationalized previously as being associated with the selective access of different collections of emissive excited states, with less states accessed at longer wavelength excitations.

Fluorescence decays of the PEI-carbon dots were measured by using the time-correlated single photon counting (TCSPC) technique (Fig. 6). The observed decays at both 400 nm and 440 nm excitations could be deconvoluted with a bi-exponential function, and the results are shown in Table 1. It should be pointed out that despite the good deconvolution fits, the excited states and processes in the carbon dots are likely more complicated than...
The average fluorescence lifetime \( \langle \tau_f \rangle = (A_1 \tau_{F1}^2 + A_2 \tau_{F2}^2)/(A_1 \tau_{F1} + A_2 \tau_{F2}) \) (see ref. 38).

For the purpose of a more direct comparison, further averaging was made by using the pre-exponential factors \( (A_1 \) and \( A_2 \) and lifetimes \( (\tau_{F1} \) and \( \tau_{F2} \) from the deconvolution fits, \( \langle \tau_f \rangle = (A_1 \tau_{F1}^2 + A_2 \tau_{F2}^2)/(A_1 \tau_{F1} + A_2 \tau_{F2}) \), and the average fluorescence lifetime \( \langle \tau_f \rangle \) values thus calculated are also shown in Table 1. These lifetime results are roughly comparable with those of the carbon dots from deliberate chemical functionalization syntheses.27,35

The spectroscopic results presented above suggest that the PEI-carbon dots obtained from thermally induced functionalization of small carbon nanoparticles by the PEI molecules are similar to carbon dots from more controlled chemical functionalization syntheses in terms of their optical transitions and fluorescence emissions, which reflect upon their associated excited state properties. From a somewhat different angle, the photo-excited state properties of carbon dots have been investigated by examining their photodynamic effects, including, for example, the use of carbon dots for photoinduced killing of cancer cells29–41 and also more recently for the visible light-driven bactericidal functions of the EDA-carbon dots.42 Thus, the PEI-carbon dots obtained from the thermally induced functionalization were also evaluated for their ability to inhibit bacterial growth upon visible light activation.

**Bacillus subtilis**, a Gram-positive bacterium, has been a popular laboratory model organism and often considered as the Gram-positive equivalent of *Escherichia coli*, an extensively studied Gram-negative bacterium.43–45 It was used in the evaluation of the visible light-activated antibacterial function of the PEI-carbon dots. Experimentally, a suspension of the cultured bacterial cells and an aqueous solution of the PEI-carbon dots were added to multiple-well plates, with the final bacterial cell concentration in each well about 10^6 CFU mL\(^{-1}\) and the concentration of the PEI-carbon dots was varied as needed (triplicates for each concentration). The plates were either exposed to visible light or kept in the dark for a pre-determined period of time. Immediately after treatment, the treated samples and the controls were serially diluted for the determination of the viable cell numbers by using the traditional plating method. The reduction in the viable cell number in the samples treated with the PEI-carbon dots and light in comparison to the controls was used as a measure of the efficiency of the light-activated bactericidal function. As shown in Fig. 7, for the sample treated with 0.02 mg mL\(^{-1}\) PEI-carbon dots, there were ~ 2.5 log viable cell reductions upon 1 h of light illumination, versus about 0.5 log reductions in the dark controls, indicating the substantial effect of visible light activation. The results are generally consistent with those from similar studies in which carbon dots from other syntheses were used.42 However, the apparently somewhat

**Table 1** Results of the deconvolution of observed fluorescence decays with a bi-exponential function

<table>
<thead>
<tr>
<th>Sample</th>
<th>( \lambda_{EX} ) (nm)</th>
<th>( \lambda_{EM} ) (nm)</th>
<th>( \tau_{F1} ) (ns)</th>
<th>( A_1 ) (%)</th>
<th>( \tau_{F2} ) (ns)</th>
<th>( A_2 ) (%)</th>
<th>( \langle \tau_f \rangle^a ) (ns)</th>
<th>( \Phi_F )</th>
</tr>
</thead>
<tbody>
<tr>
<td>As-synthesized</td>
<td>400</td>
<td>480</td>
<td>0.7</td>
<td>9</td>
<td>3.3</td>
<td>91</td>
<td>3.2</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>440</td>
<td>520</td>
<td>0.6</td>
<td>7</td>
<td>2.9</td>
<td>93</td>
<td>2.9</td>
<td>0.07</td>
</tr>
<tr>
<td>More fluorescent sample from the fractionation</td>
<td>400</td>
<td>480</td>
<td>0.9</td>
<td>8</td>
<td>4.4</td>
<td>92</td>
<td>4.3</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>440</td>
<td>520</td>
<td>0.7</td>
<td>8</td>
<td>3.7</td>
<td>92</td>
<td>3.7</td>
<td>0.12</td>
</tr>
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\(^a\) The average fluorescence lifetime \( \langle \tau_f \rangle = (A_1 \tau_{F1}^2 + A_2 \tau_{F2}^2)/(A_1 \tau_{F1} + A_2 \tau_{F2}) \) (see ref. 38).

Fig. 5 Absorption (ABS) and fluorescence (FLSC) spectra and quantum yields of the PEI-carbon dots at different excitation wavelengths (spectra shown as solid lines from left to right corresponding to excitation wavelengths from 400 nm to 560 nm in 20 nm increment). The spectra of the EDA-carbon dots (dashed lines)29 at 440 nm, 500 nm, and 560 nm excitations are also shown for comparison.

Fig. 6 Observed fluorescence decays of the as-synthesized PEI-carbon dots and the more fluorescent sample from fractionation (400 nm excitation).
significant antibacterial effect of the PEI-carbon dots even in the absence of light activation is puzzling. In addition to experimental factors such as the high sensitivity of the bactericidal function of carbon dots to even minimal ambient light exposure, the carbon nanoparticle-bound PEI species might have some surfactant-like properties, slightly inhibitive to the bacterial cell growth in the dark controls. Nevertheless, the visible light activation obviously made the PEI-carbon dots orders of magnitude more effective in the inhibition of B. subtilis. The bacterial inhibition may be attributed to the photodynamic effect, similar to what has been reported on the use of conventional semiconductor nanomaterials.56–49

Similar to carbon dots from other syntheses, the sample of PEI-carbon dots from the thermally induced functionalization in this work contained a mixture of various fractions with different fluorescence performances. The more fluorescent fractions could be harvested via separation on an aqueous gel column, as similarly practiced and reported previously. The aqueous gel column was packed in-house by using a commercially acquired Sephadex™ G-100 gel. In the fractionation, the sample of PEI-carbon dots was added to the column and eluted with water, and colored fractions were collected and characterized. The more fluorescent fractions (fluorescence quantum yields around 20% at 400 nm excitation) were combined into one sample, as the observed absorption and fluorescence spectra of the fractions were rather similar. For the more fluorescent sample thus obtained, its absorption and fluorescence spectra are similar to those of the as-synthesized sample pre-fractionation (Fig. 2). Interestingly, however, despite the significantly higher fluorescence quantum yields (Table 1), the more fluorescent sample from the fractionation exhibited fluorescence decays similar to those of the as-synthesized sample pre-fractionation (Fig. 6 and Table 1). Such a decoupling between changes in fluorescence quantum yields and decays (or average fluorescence lifetimes, Table 1) reflects upon the likely more complicated photoexcited state properties and processes in carbon dots, with significant mechanistic implications.

Mechanistically, the fluorescence emissions in carbon dots are attributed to radiative recombinations of photo-generated electrons and holes trapped at diverse surface defect sites. Experimental evidence for the involvement of electrons and holes included the results on highly efficient fluorescence quenching of carbon dots by both electron donors and acceptors, and the harvesting of the photo-generated electrons for various reactions such as the reduction of carbon dioxide into small organic molecules. Within such a mechanistic framework, the apparent decoupling between the observed fluorescence quantum yields and decays may be rationalized by the presence of two primary excited state processes following the initial photoexcitation, one for the formation (or populating) of the emissive excited states and the other for the deactivation of these states via fluorescence emissions and competing nonradiative pathways. Thus, with quantum yields for the former denoted as and those for the radiative process in the latter as , the observed fluorescence quantum yields for the combination of the two processes, , must be reflecting a combination of the first process represented by for apparently too fast to be captured in the fluorescence decay measurements, where the time resolution in terms of the instrument response function was on the order of 100–200 ps, so that the observed fluorescence decays were associated only with the deactivation process of the emissive excited states. Thus, the average fluorescence lifetimes (Table 1) are coupled with the quantum yields for the radiative pathway in the second process. In general, carbon dots with more effective surface passivation exhibited brighter fluorescence emissions and correspondingly higher fluorescence quantum yields. As such, the more fluorescent sample from the gel column fractionation was likely composed of carbon dots with more effective passivation by the surface-bound PEI species. Based on the discussion above, we can say that such enhanced fluorescence emissions and quantum yields must be primarily due to larger values. However, mechanistic details on how the improved surface passivation in carbon dots makes the process more efficient are yet to be probed and understood.

**Conclusions**

Thermally induced functionalization of pre-processed and selected small carbon nanoparticles with the oligomeric PEI yielded carbon dots with optical absorption and fluorescence properties similar to those of the dots synthesized by the deliberate chemical functionalization method. The similarity in the photoexcited state properties is also reflected in the observed visible light-activated bactericidal functions of the PEI-carbon dots. The results provide a clear validation of the hybrid approach employed for the preparation of carbon dots that combines the advantageous characteristics of the method of deliberate chemical functionalization synthesis and those of the method based on the carbonization of organic and other carbon-containing precursors. Carbon dots prepared by the deliberate chemical functionalization method are
generally nontoxic according to the results available from cytotoxicity and in vivo toxicity studies. Similar investigations on the PEI-carbon dots will be pursued.

**Experimental section**

**Materials**

The carbon nanopowder sample was purchased from US Research Nanomaterials, Inc., polyethyleneimine (PEI, branched, average molecular weight ~ 1200) was obtained from Polyscience, Inc., and silicon carbide (120 grit) was from Panadyne Abrasives. Nitric acid was obtained from Fisher Scientific and deuterated water for NMR experiments was from Cambridge Isotope Laboratories. The dialysis membrane tubes (molecular weight cut-off ~ 500 and ~ 1000) were supplied by Spectrum Laboratories. Water was deionized and purified by passing through a Labconco WaterPro water purification system.

**Measurements**

UV/vis absorption spectra were recorded on a Shimadzu UV2501-PC spectrophotometer. Fluorescence spectra were acquired on a Jobin-Yvon emission spectrometer equipped with a 450 W xenon source, Gemini-180 excitation and Tirax-550 emission monochromators, and a photon counting detector (Hamamatsu R928P PMT at 950 V). 2,6-Dimethoxyphenol (9,10-Bis(phenylethynyl)-anthrancene in cyclohexane was used as a standard in the determination of fluorescence quantum yields by the relative method (matching the absorbance at the excitation wavelength between the sample and standard solutions and comparing their corresponding integrated total fluorescence intensities). Fluorescence decays were measured in terms of the time-correlated single photon counting (TCSPC) technique on a Horiba Ultima Extreme spectrometer. The spectrometer is equipped with a SuperK Extreme supercontinuum laser source operating at 3.894 MHz repetition rate, TDM-800 excitation and TDM-1200 emission monochromators, a R3809-50 MCP-PMT detector operated at 3.0 kV in a thermoelectrically cooled housing, and FluoroHub A+ timing electronics. Analyses of the decay curves were performed by using Horiba Das6 fluorescence decay analysis software. NMR measurements were carried out on a Bruker Advance 500 NMR spectrometer. Atomic force microscopy (AFM) images were acquired in the acoustic AC mode on a Molecular Imaging PicoPlus AFM system equipped with a multipurpose scanner and a NanoWorld point probe NCH sensor. The height profile analysis was assisted by using SjPIP software distributed by Image Metrology.

**Carbon dots**

Small carbon nanoparticles were harvested from the commercially acquired carbon nanopowder sample in a procedure similar to those reported previously. In a typical experiment, the carbon nanopowder sample (2 g) was refluxed in aqueous nitric acid (8 M, 200 mL) for 48 h. The reaction mixture was cooled to room temperature and centrifuged at 1000g to discard the supernatant. The residue was re-dispersed in deionized water, dialyzed in a membrane tubing (molecular weight cut-off ~ 500) against fresh water for 48 h, and then centrifuged at 1000g to retain the supernatant. Upon the removal of water, carbon nanoparticles were recovered.

Carbon nanoparticles obtained from the above processing (100 mg) were mixed with PEI (2 g) and ethanol (1 mL) in a scintillation vial, and the mixture was sonicated (ultrasonic cleaner, VWR 250D) at 40 °C for 1 h, followed by the removal of ethanol via evaporation. Separately, a silicon carbide bath was prepared by placing silicon carbide (170 g) in a silica crucible casting dish (about 8 cm in diameter and 2.5 cm in height). The bath was pre-heated in a conventional microwave oven at 500 W for 3 min, and then the vial containing the mixture of carbon nanoparticles and PEI was immersed in the bath, followed by the microwave treatment in multiple cycles. In each cycle, the mixture in the bath was irradiated at 400 W until smoke started to appear. Upon irradiation for another 30 s, the vial containing the mixture was taken out of the bath for 1 min in the ambient temperature, and then immersed in the bath again for the next treatment cycle. After the microwave treatment of up to 30 heating–cooling cycles, the reaction mixture was cooled to the ambient temperature and dispersed in deionized water (10 mL) with vigorous sonication. The resulting aqueous dispersion was centrifuged at 20 000 g for 30 min to collect the supernatant, followed by dialysis against fresh water for 24 h. The as-synthesized sample of the PEI-carbon dots was obtained as a colored aqueous solution.

For more fluorescent PEI-carbon dots, the as-synthesized sample was separated in an aqueous gel column. The column was packed with the commercially supplied Sephadex™ G-100 gel by following the previously reported protocol. 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 under 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 until reaching about 2 cm in height, and then the column was opened for the continuous addition of the gel suspension. The gel column was washed with water until no change in the height, followed by the testing and calibration.

In the fractionation of the as-synthesized PEI-carbon dot sample, a concentrated solution of the sample was added to the gel column and eluted with water. Colored fractions (80 drops per fraction) were collected for characterization and further investigation.

**Light-activated bactericidal functions**

Fresh grown *B. subtilis* cells in nutrient broth (Fisher Scientific, Pittsburgh, PA) were washed three times with PBS and then re-suspended in PBS. 96-well plates were utilized, to each well was added 150 µL of bacterial cell suspension and 50 µL of PEI-carbon dot solution. The final bacterial cell concentration in each well was about 10⁶ CFU mL⁻¹ and the concentration of the carbon dots was varied as needed (triplicates for each concentration). The plates were either exposed to visible light (12 V 36 W light bulb) or kept in the dark for 1 h. Immediately after
the treatments, the samples were serially diluted in PBS. The viable cell numbers in the control and treated samples were determined by the traditional plating method. For each sample, aliquots of 100 μL appropriate dilutions were surface-plated on Luria-Bertani agar plates (Fisher Scientific, Pittsburgh, PA). After 24 h of incubation at 37 °C, the number of colonies was counted and the viable cell number was calculated in colony forming units per milliliter (CFU mL⁻¹) for all treated samples and the control. The reduction in the viable cell number in the carbon dots-treated samples in comparison to the control was used to evaluate the efficiency of bactericidal function of the PEI-carbon dots.

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Abstract

Semiconductor quantum dots (QDs) are known for their unique optical properties. In recent years, carbon nanomaterials of surface and/or structural defects have been found to exhibit similar properties after functionalization in various schemes. Among these carbon-based QDs are carbon dots, which are generally defined as small carbon nanoparticles with surface passivation. In fact, carbon dots now represent a rapidly advancing and expanding research field. As measured by the optical properties of carbon dots, the most effective passivation has been the surface functionalization of carbon nanoparticles with organic or polymeric molecules, corresponding to much brighter fluorescence emissions across the visible spectrum and extending into the near-IR. Therefore, carbon dots have been pursued extensively for potential bioimaging and other biomedical applications. The mechanistic framework for carbon dots includes photoinduced redox processes, similar to those found in conventional semiconductor QDs. As a result, carbon dots have also been pursued for their photocatalytic functions. In this article on surface-functionalized carbon nanoparticles or carbon dots, their representative syntheses and demonstrated properties and their potential uses as high-performance yet nontoxic fluorescence probes for bioimaging in vitro and in vivo are highlighted, so is their serving as potent photocatalysts in energy conversion applications.

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1. Introduction

Quantum dots (QDs) were originally defined as semiconductor nanocrystals of physical dimensions smaller than the exciton Bohr radius for the quantum confinement effect. Because of the quantum confinement, there is a predictable dependence of the energy gap on the nanocrystal dimension in QDs, as manifested by the corresponding variations in optical properties [1–3]. More specifically, the systematic changes in the beautiful fluorescence emission colors in semiconductor QDs such as CdSe of different sizes have generated much excitement in the research community, with extensive investigations on a variety of potential applications, especially as superior fluorescence probes for imaging and other biomedical applications [4,5]. In fact, the rationale for the use of QDs over organic dyes is now generally accepted in the literature [4,5]. Similarly bright and colorful fluorescence emissions have been found in other nanomaterials containing no conventional semiconductors, and those fluorescent nanomaterials are often referred to, more phenomenologically perhaps, as QDs as well, despite in most cases the absence of any classical quantum confinement effect. Among more popular and promising recent additions to the loosely defined QD family are carbon-based QDs, including carbon dots (Fig. 1) [6,7], graphene quantum dots [8,9], nanodiamonds [10,11], and “carbon nanotube quantum dots” [12,13]. For most of these QD-resembling carbon nanomaterials, surface functionalization is important or critical as in the case of carbon dots [14]. This is also phenomenologically similar to that in conventional semiconductor QDs, such as the surface capping of CdSe with ZnS for substantial performance improvements, despite the obvious mechanistic differences.

Carbon dots, generally defined as small carbon nanoparticles with various surface passivation schemes (Fig. 1) [6,7], have been leading the recent emergence of various carbon-based QDs, and now represent a rapidly advancing and expanding research field [15–21]. As measured by the optical properties of carbon dots, the most effective passivation scheme has been the surface functionalization of carbon nanoparticles with organic or polymeric molecules, corresponding to much brighter fluorescence emissions across the visible spectrum and extending into the near-IR [22,23]. In fact, fluorescence emissions from “naked” (no deliberate surface passivation) carbon nanoparticles in aqueous or organic suspensions have been reported in the literature, but the quantum yields are generally low to very low [24,25]. It may be argued that the surface passivation effect is provided by the solvent molecules in the suspensions [26]. The same dramatic surface passivation effect resulting in substantially enhanced optical properties has also been reported for graphene quantum dots [27–29]. Since passivation is on defects, improving defect-derived or dominated optical

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**Fig. 1.** Upper and middle: Cartoon illustrations used in the literature [22,84] on a carbon dot, generally a small carbon nanoparticle core with attached surface passivation molecules (equivalent to a soft corona). Lower: Fluorescence color variations in carbon dots corresponding to the indicated excitation wavelengths [6]. Reprinted with permission from the cited refs.
properties, it has been suggested that most graphene quantum dots of surface passivation for enhanced fluorescence emissions are essentially carbon dots of a more graphitic carbon core [14]. Therefore, in this article the focus will be on carbon dots that are surface-functionalized carbon nanoparticles, including their representative syntheses and demonstrated properties and their potential uses as high-performance yet nontoxic fluorescence probes for bioimaging in vitro and in vivo and also as potent photocatalysts in energy conversion applications.

2. Carbon dots—Syntheses and properties

Carbon dots (Fig. 1, also referred to in some literature as carbon quantum dots or C-Dots) were found originally for their bright and colorful fluorescence emissions (Fig. 2) [6,7]. Therefore, the fluorescence brightness across the visible and near-IR spectral regions has been a focus of the synthesis to produce carbon dots of the desired performance.

2.1. Functionalization of carbon nanoparticles

The original synthesis of carbon dots was based on the surface functionalization of small carbon nanoparticles with organic and polymeric molecules in established chemical reactions [6,7]. Such a deliberate functionalization approach with carbon nanoparticles as precursors has yielded some of the best-performing carbon dots in terms of their fluorescence brightness or quantum yields [22,23]. For example, Wang et al. functionalized small carbon nanoparticles from laser ablation production with the oligomeric PEG diamine (PEG1500N) for carbon dots of multicolor fluorescence emissions, particularly bright in the green [22]. The carbon nanoparticles were treated with nitric acid, which introduced oxidative moieties on the particle surface. The surface-bound carboxylic acid groups were targeted for the attachment of PEG1500N molecules under classical amidation reaction conditions. The as-prepared sample mixture was further processed by separation on an aqueous gel column, from which the most fluorescent fraction exhibited a quantum yield close to 60% (excitation at 440 nm and emissions centered around 520 nm, Fig. 2) [22]. The fluorescence performance of the PEG1500N–carbon dots is competitive to that of the commercially available CdSe/ZnS QDs both in solution and at the individual dot level for the same green spectral region (Fig. 2) [30]. In another study [31], Sun et al. demonstrated that a combination of surface doping with a wide-bandgap semiconductor such as ZnS or ZnO and PEG1500N functionalization could substantially improve the fluorescence performance of the resulting carbon dots, denoted as CZnS-Dots or CZnO-Dots, respectively. Anilkumar et al. applied the same aqueous gel column separation protocol to the as-prepared CZnS-Dots and CTiO2–Dots samples, and harvested the most fluorescent fractions from the separation that exhibited quantum yields in the same green spectral region (excitation at 440 nm and emissions centered around 520 nm) up to 78% [23].

The carbon nanoparticles used in the deliberate functionalization could come from different sources, such as the electrochemical exfoliation of graphite precursors reported by Li et al. [32]. The electrolysis was carried out in an amino-terminated ionic liquid, from which carbon dots were harvested. In some studies, carbon nanoparticles were produced from the carbonization of a carbon-rich precursor, followed by surface passivation with organic species [33–36]. For example, Choi et al. carbonized α-cyclodextrin for small carbon nanoparticles, which were functionalized by a PEG diamine and then formic acid. Even with the use of two surface passivation agents, the resulting carbon dots were not very fluorescent, with relatively low quantum yields even in the UV region [36].

![Absorbance (ABS) and fluorescence (FLSC) spectra of PEG1500N–carbon dots (--) are compared with those of Invitrogen “QD525PEG” QDs (—) in aqueous solutions (relative FLSC intensities normalized to per dot) [22].](image1)

![Photos under sunlight for solutions of the carbon dots and fluorescein (70–90% in fluorescence quantum yield) [22].](image2)

![Fluorescence microscopy images (458 nm excitation) of individual carbon dots (left) are compared with those of Invitrogen “QD525PEG” QDs (right) [30].](image3)

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Rednic et al. functionalized pre-selected and processed carbon nanoparticles with poly(N-vinylcarbazole) (PVK) thermochemically, which represents essentially a hybrid approach combining the deliberate functionalization discussed above with the carbonization processing detailed in the next section [37]. The PVK-carbon dots thus prepared were used in the fabrication of fluorescent PVK/carbon dots nanocomposites without introducing any unwanted agents or “impurities”.

2.2. “One-pot” carbonization syntheses

There have been a large number of reports on methods for the preparation or formation of carbon dots that share a common feature: the carbonization of organic or other carbon-rich/containing precursors often in “one-pot” processing [15–21]. These syntheses are generally different from the deliberate functionalization approach such that the carbon core is formed in the carbonization processing, though the use of carbon nanoparticles as precursors in thermochemical functionalization may be considered as a hybrid method in which some features of the one-pot carbonization synthesis are incorporated [37]. An early example for the one-pot synthesis was due to Peng and Travas-Sejdic, who prepared carbon dots by the dehydration of carbohydrates with strong acids and the passivation with 4,7,10-trioxa-1,13-tridecanediamine [38]. The carbonization synthesis has been extremely popular, with hundreds of literature reports on a wide variety of combinations between the carbon precursor, passivation agent, and processing scheme [15]. Particularly stunning has been the use of unusual or even bizarre precursors in the carbonization synthesis, such as hair [39], goose feathers [40], shrimp egg [41], cow manure [43], or even human urine [44]. While there is nothing unique about the carbon dots prepared with many of these selected precursors, the diverseness does suggest that carbon dots are not so “picky” with respect to the purity or exact composition of the underlying dot materials beyond the predominating carbon content. This is probably due to the fact that the photoexcited state properties of carbon dots are dictated by defects [14].

Generally speaking, the one-pot processing is convenient and versatile, but less controllable, yielding carbon dots of widely varying optical properties and performances [15]. Among the primary processing methods have been microwave irradiation, hydrothermal treatment, and thermochemical carbonization, for which some representative literature reports are highlighted as follows:

Microwave irradiation is relatively simple and quick, thus quite popular [45–48]. For example, Zhu et al. made carbon dots by heating a solution of saccharide and PEG in a microwave oven [45]. More recently, Liu et al. synthesized carbon dots of different fluorescence colors by microwave processing of poly(ethyleneimine) (PEI) and PEI+glutaraldehyde (Fig. 3) [47]. Experimentally, PEI was microwaved at 200 W (180°C) for about half an hour to yield blue fluorescent carbon dots (347 nm excitation and 464 nm emission, and quantum yield ~10%), while a mixture of PEI and glutaraldehyde (as a cross-linking agent) processed under the same conditions resulted in yellow fluorescent carbon dots (347 nm excitation and 520 nm emission, and quantum yield ~8%) [47]. Similarly, Lu et al. used a mixture of oxalic acid and urea as precursor for carbonization by microwave (700 W for about 8 min) to obtain blue fluorescent carbon dots of a relatively high quantum yield [47].

In thermochemical processing, thermal energy instead of microwave is used for the carbonization, or more accurately partial carbonization similar to that with microwave irradiation discussed above, with the surviving part of the organic precursor for passivation [49]. In the study by Stan et al. as an example, N-hydroxysuccinimide was thermally treated (180°C for about half an hour) for one-pot formation of carbon dots with blue to green fluorescence emissions [50]. The processing temperature and time were not sufficient for a complete carbonization of the precursor N-hydroxysuccinimide, with the remaining organic species to serve the function of passivation, as in many similar syntheses. In other syntheses based on the same thermochemical approach but with a slight yet significant modification to the precursor selection, multiple organic species in a mixture were used as precursor, among which one was selected to be “sacrificed” in the carbonization [35,51,52]. For example, Dong et al. used a mixture of citric

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Fig. 3. Representative schemes for the synthesis of carbon dots with the use of microwave irradiation (Upper: Ref. [45]; Lower: Ref. [47]). Reprinted with permission from the cited refs.
heating the solid-state precursor at 260 °C. Conditions were largely similar to those in other syntheses, simply varying the thermal processing discussed above, with the carbonization of organic precursor in an aqueous medium [53–55]. There have been a large number of such syntheses reported in the literature [56–59], such as the earlier investigation by Yang et al. in which carbon dots were obtained from the hydrothermal carbonization of chitosan [58], and the more recent study by Wang et al. in which blue fluorescent carbon dots were synthesized by hydrothermal carbonization of milk [59]. There have also been many reports on the use of multiple precursor organic species to divide the roles of being the carbon source and the surviving moieties for passivation in the hydrothermal carbonization processing [55,60–62].

The one-pot synthesis approach has been popular in the effort on purposely introducing heteroatoms into carbon dots [63–66]. For example, Wang et al. reported on the use of glutathione as nitrogen- and sulfur-containing precursor for the thermal carbonization into “nitrogen and sulfur co-doped carbon dots” (with nitrogen and sulfur contents of 16.1% and 2%, respectively) [66]. The processing conditions were largely similar to those in other syntheses, simply heating the solid-state precursor at 260 °C for about an hour. Wang and Zhou prepared “nitrogen-doped carbon dots” from milk [59]. Xu et al. synthesized blue fluorescent “sulfur-doped carbon dots” by hydrothermal treatment of sodium citrate solution and sodium thiosulfate in various ratios. The heteroatom doping of carbon dots apparently shares the same concept with that found in the similar modification of “graphene quantum dots” [54]. However, for carbon dots there have been so many syntheses from almost all imaginable organic, biological, and other precursor species containing a variety of heteroatoms as well as metals and other elements, and no systematic property and/or optical performance variations could be identified other than the conclusion that carbon dots are not “picky” at all in terms of elemental compositions. The studies of purposely adding heteroatoms to carbon dots have not produced any significant evidence for altering the not-picky conclusion above.

Beyond microwave and thermal, electrochemical energies have also been applied to the synthesis of carbon dots from various precursor species [32,67,68], as originally reported by Zhou et al. [25].

2.3. Host–guest carbon dots

Carbon dots from deliberate functionalization of small carbon nanoparticles can be made brightly fluorescent in the green spectral region matching that of the green fluorescence proteins, achieving fluorescence quantum yields easily higher than 50% at 400–450 nm excitation [22]. They are also fluorescent in the red to near-IR spectral regions, but the corresponding intensities and quantum yields are progressively lower [6,69]. One-pot carbonization syntheses generally yield carbon dots of relatively strong blue fluorescence, corresponding to near-UV excitation [70–72]. Those from specifically selected precursors can be fluorescent at longer wavelengths, but their quantum yields in the green and red spectral regions are mostly lower to much lower than those of the high-performance carbon dots from the deliberate functionalization approach. Therefore, the preparation of brightly red/near-IR fluorescent carbon dots with quantum yields into double digits in percentage still represents a major challenge, despite the extensive effort already made. Beyond fluorescence quantum yields, the optical absorptivity of carbon dots decreases progressively with increasing wavelength (Fig. 2), which also limits the performance of the carbon dots as fluorescence probes in the red to near-IR spectral range. As a new strategy towards the desired fluorescence probes with high performance in the biologically significant spectral window, Sun et al. recently proposed and demonstrated the development of host–guest carbon dots, which are conceptually similar to endohedral fullerenes (Fig. 4), to have red/near-IR fluorescent species enclosed in the dot structure [73]. In the reported study, fluorescent dyes cresyl violet, nile blue, and zinc phthalocyanine are selected as guests.

As discussed in the previous section, the thermal carbonization of organic precursors has been a popular approach for carbon dots, in which a portion of the precursor organic species is converted into carbon nanoparticles and the remaining serves the function of surface passivation agents. In the synthesis with microwave irradiation, there is likely the initial creation of carbonized seeds for their subsequent preferential absorption of the microwave energy toward the formation of the targeted dot structure. The microwave processing was used in the one-pot synthesis of the host–guest carbon dots, or G@CDots, where G denotes the guest fluorescent dyes cresyl violet (CV), nile blue (NB), and zinc phthalocyanine (ZnPc) [73]. Experimentally for the synthesis of CV@CDots as an example, an ethanol solution of CV was mixed well with oligomeric PEGs, followed by the removal of ethanol. The resulting mixture was irradiated with microwave until the desired carbonization
was reached. Since the hosting carbon dots are more transparent in the red/near-IR, the observed absorption spectra are generally superpositions of the host and the guest dye species (Fig. 5). According to atomic force microscopy (AFM) and transmission electron microscopy (TEM) results, these Ga@CDots synthesized from thermal carbonization reactions were still relatively narrowly distributed. Most of the dots were small, with their overall size profiles on the order of 10 nm or less (Fig. 4) [73].

Similar host–guest concept has been used for the encapsulation of magnetic elements in carbon dots [45,55,74–77]. For example, Bourlinos et al. synthesized a hybrid carbon nanostructure doped with gadolinium to be used as fluorescence–MRI dual-modality bioimaging probe [74]. Similarly, Xu et al. synthesized gadolinium-doped carbon dots from the hydrothermal carbonization of citric acid in the presence of ethylene diamine, with citric acid also as a strong chelating agent for gadolinium cations [55]. Gong et al. also prepared gadolinium-containing carbon dots via one-step microwave processing of a mixture of sucrose, sulfuric acid, diethylene glycol, and gadolinium chloride [45]. Kumar et al. applied a sonochemical method to the synthesis of carbon dots with gallium as guest, denoted as Ga@C-dots [76]. Experimentally, granule gallium was mixed with polyethylene glycol and sonicated at 50 °C with an ultrasonic transducer for about 2 h. The resulting Ga@C-dots were not so fluorescent, with quantum yield ~1% at 360 nm excitation, though they were responsive to electron paramagnetic resonance (EPR) for the purpose of photosensitization [76]. Guo et al. synthesized Ni@C-dots in a two-step process, first the hydrothermal carbonization of citric acid for carbon dots and then in the second step the carbon dots were mixed with nickel chloride and ethylene glycol, followed by the addition of hydrazine and sodium borohydride and heating at 60 °C. A characteristic feature of the Ni@C-dots was the nearly complete quenching of fluorescence emissions [77].

The host–guest carbon dots with a guest list from metal ions to organic dyes represent a highly versatile new platform in the development of carbon dots technology, promising great potential in the design and synthesis of novel dot compositions and configurations for expanded applications.

3. Optical bioimaging

Carbon dots as brightly fluorescent nanoscale probes have been pursued for their uses in fluorescence imaging in vitro and in vivo, yielding results that not only serve as the initial demonstration on their widely predicted potential but also reveal some significant challenges for the further development effort [7,15,16,78].

3.1. Cell labeling/imaging

Carbon dots are nontoxic to cells at concentration levels much higher than those commonly used in fluorescence labeling and imaging [15,16,30,79]. As reported originally by Sun et al. [6], carbon dots were readily taken up by cells, residing primarily in the cytoplasm, with only minor penetration into the cell nucleus. Many subsequent studies have demonstrated similar cell internalization of carbon dots [78,80–83], such as the work by Liu et al. in which the imaging results showed efficient uptake of carbon dots by Escherichia coli and murine P19 progenitor cells [80]. Chen et al. used carbon dots made from carbonizing sucrose with oil acid in the imaging of 16HBE cells, and they found green fluorescence emissions around the cell membrane and in the cytoplasm, but much weaker fluorescence in the cell nucleus [81]. More recently, Ruan et al. investigated the subcellular distribution of carbon dots, which were synthesized in the hydrothermal processing of spider silk [82]. The dots were found in the cytoplasm of U87 cells, but not in the endosome or mitochondria. In contrast, according to Zhang et al. [83], a conjugate containing Fe3O4 and carbon dots could be used to image the mitochondria in cells, with an enhanced cellular uptake in a magnetic field.

Carbon dots have extremely large two-photon absorption cross-sections in the near-IR, on the order of at least 40,000 Goeppert–Mayer units (1 G M = 10−50 cm4 s/photon) [7]. The two-photon excitation in the near-IR results in bright visible fluorescence emissions, which makes carbon dots excellent two-photon fluorescence probes [7,84]. Following the original report by Cao et al. [7], Tong et al. used carbon dots with amino molecules as surface passivation agent in the two-photon fluorescence imaging of HeLa cells [85]. In a similar study also on the imaging of HeLa cells, Hu et al. prepared nitrogen-doped carbon dots from alkalolamines and found that the dots internalized in the cells could be excited with two 760 nm photons for green fluorescence emissions [86].

There have been reports on carbon dots entering into the cell nucleus [87–89]. For example, Shi et al. showed that the carbon dots prepared from the hydrothermal carbonization of flower petals could be endocytosed into the cytoplasm and nucleus of A193 cells [87]. Kong et al. used carbon dots prepared from refluxing PEGs to stain cell nucleoli, suggesting that the performance was competitive to that of commercial DNA-specific dyes [88]. Fan et al. used similarly prepared carbon dots for two-photon fluorescence imaging, showing that the dots could be found around the cell membrane and in the nucleus of MCF-7 cells [89].

Carbon dots have also found uses in the fluorescence labeling of stem cells. In the more recent study [74,84], Liu et al. exploited the structural compactness of the short-chain PEG diamine-functionalized carbon dots [69] for the imaging of SD rat mesenchymal stem cells (MSCs). For both live and fixed cells, the carbon dots were found in the cell membrane and cytoplasm, though the labeling efficiency was significantly lower in the live cells (Fig. 6). The difference was attributed to the cationic surface character of the carbon dots at the biological pH, which might...
be less favorable to the uptake by the live cells. According to the results, the surface functionalities on carbon dots may play a significant role in determining the cell labeling efficiency.

Another important role of the surface functionalities in carbon dots is with their ready conjugation with species for specific targeting purposes [90–92]. For example, Yang et al. conjugated carbon dots with nuclear localization signal (NLS) peptides for fluorescence imaging of MCF-7 and A549 cells [90]. While majority of the dots were found in the cell membrane and cytoplasm, there was some accumulation within the cell nucleus. Li et al. synthesized carbon dots via the hydrothermal carbonization of glucose and sodium polyacrylate, which were designed as turn-on fluorescent probes for cancer cells that over express folate receptors [91]. In another study of targeting the folate receptors, Yang et al. prepared carbon dots by microwave heating of a folic acid - urea mixture [92]. In a comparison between HeLa cells and normal GES-1 cells, the carbon dots were largely internalized by the former but not the latter with the same incubation time, and the difference was rationalized by the folic acid species on the dot surface targeting the cancer cells [92].

In addition to mammalian cells, there have been recent studies on the use of carbon dots for fluorescence labeling and imaging of bacteria, fungi, and plant cells. For example, Nandi et al. used amphiphilic carbon dots to stain and detect bacteria based on fluorescence intensities [93]. Kasibabu et al. showed that carbon dots could stain bacterial (Bacillus subtilis) and fungal (Aspergillus aculeatus) cells in both green and red fluorescence colors [94]. In similar studies [95,96], Mehta et al. used carbon dots to stain various bacteria (E. coli, Mycobacterium tuberculosis, and Pseudomonas aeruginosa), yeast (Saccharomyces cerevisiae), and fungal (Magnaporthe oryzae). Jin et al. also stained fungal cells with carbon dots [97]. For plant cells, Wang et al. prepared oligomeric poly(ethylene glycol)-functionalized carbon dots to stain the onion epidermal cells [98].

3.2. Fluorescence imaging in vivo

The nontoxic nature of carbon dots makes them particularly valuable as probes for in vivo uses [15,99]. It was demonstrated in the fluorescence imaging experiments with mice that carbon dots are competitive in performance to the commercially supplied CdSe/ZnS QDs [99,100]. The in vivo evaluations have included the use of different injection routes. For example, Yang et al. injected carbon dots to the front extremity of mice to monitor their migration toward the axillary lymph node [99]. Following the same concept, Wu et al. used carbon dots prepared from the carbonization of honey in the effort on contrast enhancement in the auxiliary lymph node imaging [101].

Intravenous injection is important in investigations of pharmacokinetics and biodistribution, crucial to toxicological evaluations and various biomedical applications of carbon dots. In the early investigation [99], carbon dots were found to be excreted via urine rather efficiently, without any major accumulation in the internal organs, likely due to their small sizes (on the order of 5 nm in diameter) and high solubility. Nevertheless, in a more recent study Li et al. managed to use intravenously injected carbon dots for ex vivo fluorescence imaging of the slices from heart, liver, spleen, kidneys, lungs, brain, and small intestine [78].

Significant effort has been made to tailor carbon dots for targeting tumors in vivo. For example, He et al. attached arginyl-glycyl-aspartic acid to carbon dots for the targeting of HeLa tumors in mice [102]. Upon intravenous injection, fluorescence signals could be detected at the tumor site and in the bladder. The results from the imaging of dissected organ specimens were similar, with
only weak fluorescence found in liver, heart, spleen, and kidneys, consistent with the finding in the early study using carbon dots without the specific targeting moieties [99,102]. Skarker et al. carbonized hyaluronic acid in a dehydration reaction with sulfuric acid to yield two kinds of carbon dots, denoted as HA–FCN and FCN in which the precursor hyaluronic acid was partially and nearly completed carbonized, respectively, with the former upon intra-venous injection exhibiting more accumulation at the tumor site [103]. Wang et al. used carbon dots in the fluorescence imaging of glioma (Fig. 7) [104]. Fu et al. embedded carbon dots in silica nanorattle to be used as fluorescence probes for tumor imaging [105].

Zhou et al. prepared cholera toxin B conjugated-carbon dots (CTB-CDs) as a fluorescent retrograde neural tracer [106]. Upon the injection of CTB-CDs on the back of a mouse, blue fluorescence was recognizable. However, the desired noninvasive in vivo imaging was hindered by the strong autofluorescence due to the short excitation and emission wavelengths associated with CTB-CDs. For ex vivo imaging, CTB-CDs were injected to the mid-thigh level of the right sciatic nerve, and then the tissues were sliced. Strong fluorescence signals from CTB-CDs were observed in the ipsilateral sciatic nerve, and the accumulation of the probes at the tumor site were detected, and the laser excitation of the probes in the mice could significantly suppress the tumor growth [110].

There have been studies on the conjugation of carbon dots with magnetic species for dual-modality imaging in vivo. Srivas-tava et al. fabricated iron oxide-doped carbogenic nanocomposite (IO-CNC) for fluorescence–magnetic resonance (MR) imaging [111]. After intravenous injection, spleen tissue samples were collected, in which fluorescence signals from IO-CNC were observed. Separately in the MRI imaging, there were enhanced signals in the brain blood vessel under both T1 and T2 models [111]. More recently, Xu et al. used gadolinium-in-carbon dots as probe for MRI study of time-dependent biodistribution in mice [55].

Carbon dots are generally more fluorescent in the green than in the red/near-IR, but the latter is more favorable to tissue transmittance in imaging applications. In the work by Huang et al. [109], brightly green fluorescent carbon dots were linked with the fluorescence dye ZW800 for its strong emissions in the red/near-IR, and the resulting probes were used for imaging in vivo and ex vivo. The ZW800-linked carbon dots were efficiently and rapidly excreted from the body after injection in different routes. Post intravenous injection, there were some probes found in liver, spleen, and lungs within an hour, and very bright fluorescence was observed in kidneys and the urine excretion was confirmed. All injection pathways led to meaningful tumor uptakes [109]. For another configuration of the carbon dots–dye FRET probes to extend the emission color to longer wavelengths, Huang et al. attached the fluorescence dye Ce6 to carbon dots to allow blue excitation (430 nm) and red fluorescence emissions (668 nm) via FRET [110]. After intravenous injection, the accumulation of the probes at the tumor site were detected, and the laser excitation of the probes in the mice could significantly suppress the tumor growth [110].

Kang et al. used zebra fish as a model for the evaluation of carbon dots [112]. Upon soaking for 3 h, the carbon dots entered into embryos across the chorion and the germ ring. The fluorescence imaging of the dots allowed the visualization of the embryo development. Similarly, Fahmi et al. prepared phenylboronic acid-modified magno-fluorescent nano-probes, which consisted of MnFe2O4 nanoparticles conjugated to carbon dots, for the imaging of zebra fish [113]. Strong fluorescence emissions from carbon dots were observed in the brain, nervous system, and muscles of the fish embryo. Shi et al. hydrothermally treated petals to produce carbon dots for the imaging of carp fish [87]. Yuan et al. used carbon dots in the fluorescence imaging of Caenorhabditis elegans [114].
incubation, the C. elegans was homogenously labeled by the carbon dots, with blue or green fluorescence emissions corresponding to different excitation wavelengths. Parvin et al. prepared carbon dots that are polyelectrolyte-like for the imaging of Drosophila [115]. Drosophila melanogaster was incubated with the dots for the imaging at different developmental stages (larvae, pupa and adult). At 12-h post-incubation, for example, internal organs of larva could be clearly recognized [115].

3.3. Theranostics

Beyond imaging, carbon dots have been studied for potential uses in theranostics, namely for the concurrent effect of imaging and treatment such as drug delivery or photodynamic therapy [116–126]. For example, Lai et al. prepared carbon dots from glycerol inside mesoporous silica nanoparticles for both fluorescence imaging and drug release [119]. Ding et al. synthesized carbon dots in hydrothermal carbonization of DNA, and then loaded the anticancer drug doxorubicin (DOX) through presumably π–π stacking interactions [120]. The drug-loaded carbon dots were incubated with S. cerevisiae, and the results from microscopy imaging suggested intracellular drug release. Wang et al. also used carbon dots to carry DOX for both imaging and delivery, with the imaging as a tool for the evaluation of the delivery [121]. Carbon dots were found in the cytoplasm of human breast cancer MCF-7, MDA-MB-231, and BT-549 cells. However, the MTT assay results suggested that the toxicity of the DOX carried by carbon dots was lower than that of free DOX, which was attributed to the slow release of DOX from carbon dots [121]. Matai et al. prepared a hybrid of carbon dots and PAMAM dendrimer for the imaging and delivery of the anticancer drug epirubicin [122]. For both MCF-7 and NIH 3T3 cells, the toxicity of the hybrid carried epirubicin was somewhat lower than that of free epirubicin. Palashuddin et al. prepared carbon dots-like nanoparticles embedded with Cu2+ [123]. In HeLa cells, blue fluorescence from the nanoparticles was observed. While the nanoparticles without Cu2+ were nearly nontoxic, they apparently enhanced the toxicity of the embedded Cu2+ to HeLa cells, for which a suggested possible mechanism was such that the nanoparticles up-regulated the sub G1 population of cells and induced apoptosis [123]. More recently, Cheng et al. conjugated carbon dots with polycation-b-polyzwitterion copolymers and used the resulting conjugate as a serum-resistant gene delivery carrier to be compared with the widely used carrier PEI25K [124]. The carrier was loaded with DNA vectors for green fluorescent proteins (GFP) and transfected into COS-7 cells at different serum concentrations. It was found that the carrier was better in performance than PEI25K, especially at high serum concentrations [124]. Wang et al. used carbon dots–polyethyleneimine complexes to deliver siRNA to gastric cancer cells (Fig. 8) [125], while Hu et al. hydrothermally carbonized polyethyleneimine into carbon dots for gene delivery to take advantage of the dots being positively charged [126].

The photoactive nature of carbon dots makes photodynamic therapy a natural choice for combination with fluorescence imaging, and a number of studies have been reported for such an approach in theranostics [36,127–129]. For example, Choi et al. prepared carbon dots with both PEG diamine and folate acid as surface passivation agents and then loaded with zinc phthalocyanine (ZnPc) for imaging and photodynamic therapy [36]. Upon the incubation with HeLa cells, the ZnPc-loaded carbon dots were internalized, exhibiting blue–green and red fluorescence emissions assigned to the carbon dots and ZnPc at 358 nm and 647 nm excitations, respectively. The cells were irradiated with 660 nm laser
light (30 mW/cm²), which reduced the cell viability to 10% [36]. Kleinaukas et al. demonstrated that the silver-doped carbon dots could serve as sensitizers in photodynamic therapy and radiotherapy [127]. Wang et al. prepared nanoparticles containing carbon dots, Fe₃O₄, and gold for magnetic/near-IR-responsive drug release, multicolor fluorescence imaging, and photothermal therapy [128]. For the nanoparticles in B16F10 cells, blue, green, and red fluorescence emissions could be detected with 405 nm, 488 nm, and 546 nm excitations, respectively, and the emissions were stable under the imaging conditions. When the nanoparticles were used to carry the anticancer drug DOX, the release was enhanced in a magnetic field and by near-IR irradiation. Photothermal effect in the nanoparticles was confirmed, and its combination with the DOX delivery and release resulted in a high toxicity against B16F10 cells [128]. To take advantage of the large two-photon absorption cross-sections of carbon dots, Wang et al. linked porphyrins to carbon dots for two-photon excitation of the dots at 700 nm and then energy delivery and release resulted in a high toxicity against B16F10 cells [128]. To take advantage of the large two-photon absorption cross-sections of carbon dots, Wang et al. linked porphyrins to carbon dots for two-photon excitation of the dots at 700 nm and then energy transfer (FRET) to the porphyrins for photodynamic effect [129].

The use of carbon dots as antioxidant in addition to fluorescence imaging has also been pursued. For example, Das et al. compared the antioxidant functions of carbon dots with those of typical molecular antioxidants such as butylated hydroxytoluene and L-ascorbic acid [130]. Similarly, Zhao et al. reported on the free radical scavenging activity of carbon dots, which were prepared by the hydrothermal treatment of garlic [131].

Among the studies on carbon dots for theranostics in vivo, Choi et al. prepared PEGylated carbon dots with or without the functionalization of formic acid, and then loaded with zinc pthalocyanine [36]. In the in vivo biodistribution and photodynamic therapy experiments with tumor-bearing mice, fluorescence signals from the dots without formic acid were detected mainly in liver, much weaker in kidneys, intestine, and spleen. For the dots with formic acid, the fluorescence results suggested an appreciable accumulation in the tumor. At 12-h post-injection, the irradiation with 660 nm laser (0.3 W/cm²) for 20 min resulted in a 4-time reduction in the tumor volume [36]. Ge et al. used carbon dots for fluorescence and photoacoustic detection and thermal theranostics in mice (Fig. 9) [132]. After intravenous injection, fluorescence from the carbon dots could be detected in the tumor area. The imaging ex vivo suggested that the dots were mostly in the liver and tumor, very minor in heart, spleen, and lungs. Photoacoustic signals were also found in the tumor area. The tumor site with the dots was illuminated with a near-IR laser for 3 and 10 min, resulting in temperature increases at the site to 50.4 °C and 60 °C, respectively. The photothermal therapy induced substantial empyrosis and suppressed the tumor growth [132].

Liu et al. used the conjugate of carbon dots with ribonuclease A for synchronous cancer imaging and therapy [133]. The conjugate could be found in both cell cytoplasm and the nucleus, and it exhibited higher toxicity to MGC803 cancer cells than free ribonuclease A. Upon the intratumoral injection of the conjugate, bright fluorescence was detected at 10 min and 4-h post-injection, but the signal decreased dramatically after 12 h [133]. Tang et al. prepared the conjugate of carbon dots with folic acid and the anticancer drug DOX for theranostics [134]. Glomerular tissue incubated with the conjugate was imaged by using a 3D two-photon fluorescence microscope to monitor the release of DOX.

4. Photocatalytic energy conversion

Carbon dots resemble conventional semiconductor QDs beyond the similar bright and colorful fluorescence emissions. They also share the characteristic behavior of driving photocatalytic energy conversion processes, such as the photoreduction of CO₂ into small organic molecules [135–137].

4.1. Photoinduced redox processes

In the presently adopted mechanistic framework, the photoexcitation of carbon dots results in efficient charge separation, with the separated electrons and holes (or radical anions and cations in a different description) trapped at various surface sites that are passivated by the surface functionalization species, and the radiative recombination of the electrons and holes are responsible for the observed fluorescence emissions (Fig. 10) [14,138]. This is in several respects similar to the photoexcited state mechanism in conventional semiconductor QDs [4,5]. The mechanistic framework for carbon dots has been supported by experimental results [138–140], including especially those demonstrating that photoexcited carbon dots are both excellent electron donors and acceptors, with fluorescence emissions quenched efficiently by electron acceptor and donor molecules statically and dynamically in a diffusion-controlled fashion (Fig. 10) [138]. Within the mechanistic framework, the fluorescence quenching results could readily be explained in terms of the electron acceptor or donor quenchers scavenging the electrons and holes in carbon dots, respectively, thus disrupting the radiative recombinations (Fig. 10). In a subsequent investigation using the same electron acceptor and donor quenchers as those in ref. 138, 2,4-dinitrotoluene and N,N-diethylaniline, respectively, Zhang et al. performed fluorescence decay measurements to have the results confirm the redox processes in photoexcited carbon dots [139]. More recently, there was a study based on transient absorption spectroscopy to probe more directly the redox species and processes associated with photoexcited carbon dots [141]. Triethanolamine (TEOA) and methyl viologen (MV²⁺) were used as electron donor and acceptor, respectively, for redox interactions with the carbon dots.

In other recent studies, Mondal et al. synthesized hydrophobic carbon dots via thermal carbonization of glucose in the presence of dodecylamine, and then loaded cyclometalated complexes of Ir(III) and Rh(III) onto the dot surface in a nonpolar solvent gradient [142]. Upon photoexcitation of the carbon dots loaded with the metal complexes, there was fluorescence quenching, more significant at a higher loading of the metal complexes. The quenching was attributed to electron transfer or more specifically hot electron injection from the metal complexes into the excited carbon dots [142]. Mondal et al. also used amino acids as precursor for carbon dots, with dot surface protected by surfactants of different chain lengths from 12 to 16 carbons [143]. The fluorescence emissions of these carbon dots were quenched by dimethylaniline as electron donor. A somewhat surprising observation was that the quenching efficiency was higher for carbon dots protected by a longer chain surfactant [143]. In a similar study of photoinduced electron transfer, Gao et al. found that fluorescence emissions of the carbon dots could be quenched by Fe(III) [144].

The surface-bound electrons generated from the photoexcitation of carbon dots could be used to reduce metal ions in solution to result in the coating of the carbon dots with the corresponding metal [140,145]. For example, the photoirradiation of carbon dots in an aqeous solution of gold compound was used to deposit gold metal on the dot surface, which was accompanied by an extremely efficient static quenching of fluorescence emissions [140]. Since the metal is electron affinitive, it takes electrons from the attached carbon dots, thus disrupting the radiative recombinations. The photo-reductive deposition of a noble metal on carbon dots has valuable applications, such as much improved photocatalytic functions [146,147]. Choi et al. also exploited the same processing to decorate the surface of carbon dots with plasmonic silver nanoparticles for hybrid nanostructures of enhanced light harvesting capability in optoelectronic devices (Fig. 11) [145]. The observation on effective quenching of fluorescence in carbon dots by the presence of silver was also reported [148]. Similarly,
Mazzier et al. exploited the electron-donating character of pho- 
toexcited carbon dots to grow silver nanoparticles on the surface 
of carbon dots [149]. The results again served as experimental 
evidence for the availability of photo-generated electrons in carbon 
dots that could be harvested for reduction or other productive 
purposes.

4.2. Photocatalytic functions

One of the most challenging yet rewarding photocatalytic 
processes for energy conversion is the reduction of CO₂ into 
small molecular fuels, with solar radiation in particular. Semi-
conductor nanomaterials, including especially colloidal TiO₂ have 
traditionally been employed as photocatalysts in the relevant reac-
tions. However, TiO₂ nanoparticles and other semiconductor QDs 
for such a purpose are absorptive only or mostly in UV, inefficient 
in the harvesting of solar photons, so that a number of strategies 
to extend the absorption into the visible spectrum have been pur-
sued [150]. Carbon dots are broadly absorptive over the UV and 
visible spectral regions, extending into the near-IR, with a signifi-
cant overlap with the solar spectrum (Fig. 12). The photoexcitation 
in carbon dots drives the excited state redox processes, which are 
not only responsible for the observed fluorescence emissions but 
also make the associated electrons and holes available for the cata-
lytic energy conversion reactions, including the CO₂ reduction 
[147].
It has been demonstrated that carbon dots with surface passivation by amino or other molecules are capable of serving as photocatalysts with broad-band visible excitation for CO$_2$ reduction in aqueous solution (Fig. 13)\cite{135,137}. Experimentally, the carbon dots were irradiated with light over a broad visible spectral range (405–720 nm) in an aqueous solution saturated with CO$_2$ (or NaHCO$_3$ as the CO$_2$ source), with the reduction monitored by the detection and quantification of formic acid as a major product. The formic acid formation was apparently significant, around 40 $\mu$mol g$^{-1}$ h$^{-1}$, better than the performance with the use of colloidal TiO$_2$ (Degussa P25) as photocatalyst and UV irradiation\cite{137}. The results of photocatalytic CO$_2$ conversion have also contributed to the understanding of the mechanistic framework, providing valuable experimental evidence for the photo-generated redox species in carbon dots, as already suggested by the fluorescence quenching results (Fig. 10)\cite{138,140,145}. Similarly of mechanistic values was the much improved photocatalytic performance of the carbon dots after the photo-deposition of gold on the dot surface (Fig. 13)\cite{33,135,137}, which in terms of the mechanistic framework must be due to the concentration of...
the photo-generated electrons by the gold metal [150]. For the gold-coated carbon dots as photocatalysts in an aqueous solution saturated with CO2 and the photoirradiation with light over a broad visible spectral range, the estimated quantum yield for the conversion to formic acid was \( \sim 0.3\% \) [135]. This likely underestimated the actual overall quantum yield for the CO2 photocatalytic reduction because there are other photoproducts in addition to formic acid.

The relatively efficient conversion to formic acid is in itself a reflection on the effectiveness of the metal-coated carbon dots as photocatalysts. Beyond formic acid, other photoproducts have been identified, in which the characterization and quantification of acetic acid are particularly significant [136], as the photoreduction of CO2 to acetic acid requires overall eight electrons, regardless of mechanistic details [150]. In fact, the proposed mechanisms in the literature on the conversion to acetic acid had to invoke the involvement of other small organic molecules as intermediate products, especially methanol [136,150–152]. Therefore, not only the overall CO2 conversion quantum yields must be higher than that for only the formic acid formation, but also the results on acetic acid and methanol as other significant products in addition to formic acid suggest that carbon dots represent uniquely potent broad-band visible-light photocatalysts for the CO2 conversion.

Other noble metals have been used to coat carbon dots, and the resulting “hybrid nanostructures” have exhibited similarly potent photocatalytic functions. For example, the results from the use of platinum-coated carbon dots as photocatalysts in the CO2 conversion were largely the same as those with their gold-coated counterparts [135]. Li et al. prepared nanocomposites of carbon dots with Cu2O as visible-light photocatalysts for the conversion of CO2 into methanol [153]. The photoinduced redox properties of the carbon dots/Cu2O nanocomposites were examined in the fluorescence quenching study with the known electron acceptor 2,4-dinitrotoluene and electron donor N,N-diethylaniline as redox quenchers [153]. The quenching results suggesting the presence of photo-generated redox species in the carbon dots/Cu2O nanocomposites were used to justify their photocatalytic functions in the CO2 conversion. The proposed mechanism was such that the visible-light excitation of Cu2O produces electron-hole pairs, with the electrons consumed for the CO2 reduction and the holes transferred to the surface of the carbon dots for the oxidization of H2O into O2 [153].

![Fig. 12](image_url) The observed absorption spectrum of broadly distributed carbon dots in aqueous solution (solid line) compared with the solar spectrum at the sea level (dashed line). Reprinted with permission from ref. [147].

Fig. 13. Cartoon illustrations on (upper) the high-pressure optical reactor; (lower-left) the photoreductive doping of the carbon dot with gold, completely quenching the dot surface-based fluorescence (illustrated as the change of the dot surface from rainbow to grey); and (lower-right) the gold-doped carbon dot as photocatalyst for CO2 conversion, where the doped gold (in yellow) was small in quantity, insufficient to form a shell, and likely random in terms of size and shape. The sacrificial electron donor was isopropanol added in some experiments or PEG1500 molecules on the surface of the carbon dots in the absence of isopropanol. Reprinted with permission from ref. [136].
Particularly interesting and consequential results on the use of carbon dots and their metal-coated hybrid nanostructures as photocatalysts for the conversion of CO₂ into useful molecules have been reported. These findings reflect an ever-increasing number of recent publications. Some of this research is still experiencing rapid advances, as evidenced by the emergence of new methodologies, such as the use of nanoparticles, which have proven to be important tools.

5. Summary and perspectives

Carbon dots, which are generally functionalized carbon nanoparticles, have obviously emerged to represent an important research field that is still experiencing rapid advances, as reflected by an ever-increasing number of recent publications. Some characteristics of this research field, recent trends, and possible future directions are summarized as follows.

There have been a disproportionately large number of publications on the synthesis of carbon dots, mostly by a partial carbonization of a variety of carbon-rich or carbon-containing precursors. The relevant methodologies are generally simple and versatile, but the resulting carbon dots have yet to reach the performance levels achieved by carbon dots obtained from the deliberate functionalization of carbon nanoparticles, and an understanding of the structural details in the dots from the carbonization synthesis still presents a significant challenge. Hybrid approaches that combine the deliberate functionalization concept with the facile carbonization processing may prove valuable in the versatile and efficient preparation of carbon dots with different surface functionalities and in large quantities.

The increasing amount of experimental evidence for the nontoxic nature of carbon dots will have far-reaching implications in their further development, stimulating more explorations that target in vivo and ex vivo uses in biology and medicine. In addition to the fluorescence imaging emphasized in this review, carbon dots have also been explored for their electrochemiluminescence properties [154–156] targeting related imaging applications [157,158]. With the performance benchmarks already achieved, carbon dots are expected to find biomedical applications that have been widely pursued in the research field of semiconductor QDs. Special opportunities are in those imaging-sensing and related uses that require human interactions, in vivo, ex vivo, food, water, and so on, to take advantage of the unique attributes of carbon dots as being high-performance yet benign and nontoxic. Significant advances in areas such as carbon dots as a new platform for multi-modality imaging agents and for combining imaging with drug delivery and therapy may be envisaged.

Beyond bioimaging and theranostics, there has been increasing recent attention on the photocatalytic functions and related properties of carbon dots. Carbon dots have also been explored for photoelectrochemical [159,160] and optoelectronic applications [145,161,162]. These are hardly surprising considering the fact that carbon dots share some of the key mechanistic steps with conventional semiconductor QDs, namely carbon dots are essentially nanoscale semiconductors in many respects. Therefore, it may be expected that investigations into the relevant photoinduced redox properties of carbon dots and their uses in photocatalytic, photoelectrochemical, and optoelectronic processes and devices will continue and be expanded significantly.

Lastly, there have been some confusions on the relationships between different carbon-based QD-like nanomaterials, carbon dots vs graphene quantum dots in particular [14,147]. While such confusions may take some time to resolve, there is the prospect for a unified and mechanistically consistent understanding of these nanomaterials when more experimental as well as theoretical results become available. As a takeaway from this article, it is the surface functionalization of carbon nanoparticles that makes carbon dots and their associated superior optical and other properties, and the same functionalization effect is apparently evident in the field of graphene quantum dots (and their various structurally heteroatom-doped derivatives [147]), likely a reflection of their shared mechanistic origins. Further investigations are obviously needed.

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Visible-Light-Activated Bactericidal Functions of Carbon “Quantum” Dots

Mohammed J. Meziani,†‡ Xiuli Dong,‡∥ Lu Zhu,§∥ Les P. Jones,¶ Gregory E. LeCroy,† Fan Yang,† Shengyuan Wang,‡ Ping Wang,‡ Yiping Zhao,‡⊥ Liju Yang,‡⊥ Ralph A. Tripp,†∥ and Ya-Ping Sun*†

†Department of Chemistry and Laboratory for Emerging Materials and Technology, Clemson University, Clemson, South Carolina 29634, United States
‡Department of Pharmaceutical Sciences, Biomanufacturing Research Institute and Technology Enterprise, North Carolina Central University, Durham, North Carolina 27707, United States
§College of Engineering, ¶Department of Infectious Diseases, and †Department of Physics and Astronomy and Nanoscale Science and Engineering Center, University of Georgia, Athens, Georgia 30602, United States

Supporting Information

ABSTRACT: Carbon dots, generally defined as small carbon nanoparticles with various surface passivation schemes, have emerged as a new class of quantum-dot-like nanomaterials, with their optical properties and photocatalytic functions resembling those typically found in conventional nanoscale semiconductors. In this work, carbon dots were evaluated for their photoinduced bactericidal functions, with the results suggesting that the dots were highly effective in bacteria-killing with visible-light illumination. In fact, the inhibition effect could be observed even simply under ambient room lighting conditions. Mechanistic implications of the results are discussed and so are opportunities in the further development of carbon dots into a new class of effective visible/natural light-responsive bactericidal agents for a variety of bacteria control applications.

KEYWORDS: carbon dots, bactericidal functions, light activation, photodynamic effect, E. coli, colloidal TiO₂

INTRODUCTION

Bacterial infections present a major healthcare challenge, especially with the increasing bacterial resistance to antibiotics,1,2 demanding the development of alternative antimicrobial strategies. Among the most effective alternatives is the use of photoactivated antimicrobial nanomaterials and nanotechnology, for which the recent rapid advances offer some unique opportunities. Nanoscale semiconductors have been a popular choice for their photoinduced redox properties and associated bactericidal functions. For example, colloidal TiO₂ has been widely employed as a photocatalyst for antibacterial and general disinfection purposes.3 However, a significant limitation with TiO₂ nanostructures is the large band gap (3.2 eV), requiring UV activation. Therefore, there has been much effort on the modification of TiO₂ via doping or coupling with dyes or narrower-band-gap materials to extend photoexcitation into the visible spectrum.4–6 Alternative semiconductors and other nanomaterials for visible-light-responsive antibacterial activities have been pursued.7–9 Visible-light activation considerably broadens the reach of the photochemical antimicrobial agents, potentially enabling their uses under solar irradiation or natural-light exposure to inhibit the growth of pathogens and other infectious agents. Our focus has been on exploring the newly developed carbon “quantum” dots, or more appropriately called carbon dots for their lack of classical quantum confinement behavior,10 for their visible-light-activated bactericidal functions.

Carbon dots (Figure 1),10 generally defined as small carbon nanoparticles with various surface passivation schemes,11 have emerged as a new class of quantum-dot-like nanomaterials, with their optical properties and photocatalytic functions resembling those found in conventional nanoscale semiconductors.11–17 For example, in addition to their bright and colorful fluorescence emissions, carbon dots have been demonstrated as effective visible-light photocatalysts for oxidation and reduction reactions.17,18 The same photoinduced redox processes responsible for the photocatalytic activities should make carbon dots excellent candidates as antibacterial agents, for which a major advantage is the broad and strong optical absorption of carbon dots over the visible spectral region, extending into the near-IR. Here we report the experimental confirmation on the bactericidal functions of photoexcited carbon dots.2,2’-(Ethylenedioxy)bis(ethylamine) (EDA) carbon dots were selected for being compact in structure and well-
characterized in a previously reported study.19 The evaluation experiments were performed under household LED lighting or ambient laboratory light conditions, and the carbon dots inhibited *Escherichia coli* cells in all of the experimental settings. These results and their mechanistic implications are discussed.

**RESULTS AND DISCUSSION**

Carbon nanoparticles as precursors to carbon dots were obtained from the commercially supplied carbon nanopowder sample by following an established protocol including the refluxing of the as-supplied sample in an aqueous nitric acid solution, dialysis, centrifuging to retain the supernatant, and then drying to recover the carbon nanoparticles. The nanoparticles were surface-functionalized with EDA under amidation reaction conditions to yield EDA carbon dots.19,20 Briefly, the carbon nanoparticles were refluxed in neat thionyl chloride, followed by the removal of excess thionyl chloride, and then the sample was mixed well with carefully dried EDA. The mixture was heated and stirred at 120 °C with nitrogen protection in the experimental setup designed for reactions under refluxing conditions. The reaction mixture was dispersed in water and centrifuged to retain the supernatant as the as-synthesized sample, which was further purified by removing residual small molecular species including free EDA in dialysis against deionized water to obtain EDA carbon dots in an aqueous solution. The solution appeared optically transparent (Figure 2), stable without any precipitation over an extended period of time (many months). The solubility and solution stability may be expected for these dots, being small carbon nanoparticles with the surface well-functionalized by hydrophilic molecules (Figure 1). According to results from atomic force microscopy (AFM) and transmission electron microscopy (TEM) characterization, the EDA carbon dots were on the order of 5 nm diameter (Figure 3).

The optical absorption of carbon dots is due to a π-plasmon transition in the carbon nanoparticle core, with a broad absorption spectrum covering most of the visible region (Figure 2). The carbon dots in aqueous solution are brightly fluorescent, with the emission colors dependent on the excitation wavelengths (Figure 2), which along with the broad fluorescence spectra suggests a distribution of emissive excited states. It is known in the literature that the fluorescence emissions of carbon dots could be quenched effectively with either electron donors or acceptors, supporting the notion that the redox characteristics in the photoexcited states of carbon dots are responsible for their observed photocatalytic activities.11,21,22 The same characteristics were exploited in this study for their visible-light-activated bactericidal functions.

![Figure 1. Cartoon illustrations of (left) a carbon dot, with a small carbon nanoparticle core and the surface functionalization molecules forming a soft shell, and (right) the photoexcited-state species and processes, with the rainbow color showing fluorescence from the dot surface.](image1)

![Figure 2. Top: Absorption spectrum of the EDA carbon dots in an aqueous solution (photograph in the inset). Bottom: Fluorescence spectra of the EDA carbon dots in an aqueous solution excited at the indicated wavelengths.](image2)

![Figure 3. TEM (top) and AFM (bottom) images of the EDA carbon dots on commercial TEM grid and mica, respectively.](image3)
E. coli (K12) cells were used in the experiments to evaluate the antibacterial activities of photoexcited carbon dots, with the cell growth measurements based on the optical density (OD) at 600 nm (OD600) and/or the viable cell number determined by the plating method. Experimentally, the E. coli cells were inoculated in a 12-well plate at 0.2 OD/mL per well, treated with the EDA carbon dots of different concentrations, and exposed to ambient light for an hour in a safety cabinet. Then, the plate was incubated for 21 h, followed by the measurement of OD600. As shown in Figure 4, the results clearly suggest that there were substantial effects of the EDA carbon dots with light exposure on the E. coli cells. In the literature, there was a report on some antibacterial activities of the carbon dots obtained from carbonization of glucose and poly(ethyleneimine), with the dots quaternized with benzyl bromide before bacteria experiments.23 While no deliberate light exposure was mentioned in that report, the ambient experimental conditions could have contributed to the reported observations.

Similarly, the E. coli cells in aqueous suspension were mixed with an aqueous solution of EDA carbon dots, and the resulting mixture containing ~10⁷ colony-forming units per milliliter (CFU/mL) E. coli was incubated for 30 min at room temperature under visible-light illumination (12 V, 36 W bulb in a light box) or in the dark as the control. Then, the growth of E. coli cells after treatment with and without light exposure was monitored. Shown in Figure 5 are the growth curves of E. coli in a brain heart infusion medium post-treatment with EDA carbon dots, along with the control (untreated cells), based on OD measurements at 595 nm (OD595). The treated cells exhibited much prolonged lag phases (7–8 h) compared to the control, indicating that the EDA carbon dots inhibited/inactivated the growth of bacterial cells. The effect was somewhat more pronounced (the lag phase was longer by 1 h) when the treatment included exposure to visible light (Figure 5), but the light versus dark inhibition difference was not as obvious as that shown in Figure 4 (for which the experiments were performed in different laboratories at different times). Therefore, to address the inconsistency issue at the quantitative level for the OD measurement method, separate experiments with the same parameters and conditions were performed for the inhibition effect probed by the more quantitative method of determining the viable cell numbers post-treatment.

Again the samples of E. coli with and without (control) EDA carbon dots were treated in the light box or in the dark for 30 min. The viable cell numbers in the treated samples were determined, and according to the results (Figure 6), the EDA carbon dots treatment coupled with visible-light illumination was obviously effective for bacteria killing, with about 4 logs of E. coli cells killed. Compared to the results shown in Figure 4, the bacteria-killing effect seemed more dramatic in these experiments. A significant contributing factor might be the use of a lamp instead of ambient light for the photoexcitation of carbon dots, although more systematic and quantitative experiments are needed in further investigation. Nevertheless, the results are all consistent in terms of confirming the visible-light-activated bactericidal functions of EDA carbon dots.

A different experimental configuration was employed for further evaluation on the bactericidal activities of EDA carbon dots, in which the dots were plated on the trypticase soy agar (TSA) plates with bacteria cells during visible-light exposure. In a typical experiment, an E. coli (TOP10) suspension of 1.3 × 10⁷ CFU/mL concentration was mixed with an aqueous solution of EDA carbon dots, and the mixture was spread onto the TSA plates. Upon exposure of the plates to visible light (Osram Sylvania LED A19 lamp, ~10 mW/cm²) for up to 6 h, there were obvious differences between the treated plate and controls (Figure 7), again suggesting a substantial bactericidal effect of EDA carbon dots with visible-light illumination. The plates were read for CFU counts, and according to the results, the difference was not as obvious as that reported in the other experiments, which might be attributed to the use of another light source or experimental conditions. Further experiments are needed to investigate this difference in detail.
photoexcited carbon dots were very effective in inactivating the growth of bacteria cells (Figure 7).

The results presented above, which were obtained in different laboratories of the participating research groups under various experimental settings, provide consistent and unambiguous evidence for the highly effective bactericidal functions of carbon dots under visible-light illumination, including even the common household lighting conditions. Mechanistically, carbon dots have been demonstrated for photodynamic effects on cancer cells, and similar effects on bacterial cells might be a logical extension. The current mechanistic framework for the known optical properties of carbon dots is such that upon photoexcitation there are efficient charge separations for the formation of radical anions and cations (electrons and holes in a somewhat different description), which are "trapped" at various passivated surface sites. The radiative recombination of redox pairs is responsible for the observed fluorescence emissions, with their associated emissive excited states of lifetimes on the order of a few nanoseconds. The radiative recombination of redox pairs is responsible for the observed bactericidal functions. However, in the fluorescence decay measurements, the rise time for the fluorescence was generally within the instrumental response function (1 ns or less), suggesting rather fast radiative recombination and short lifetimes of the radical-ion species. Therefore, the emissive excited states are more likely responsible for the photodynamic effects.

There have been no reports in the literature that explicitly describe the apparently effective bactericidal functions of photoexcited carbon dots. As related, there have been a few studies of using "graphene quantum dots" as photodynamic agents. In the study by Ristic et al., antibacterial activities were observed with 470 nm light irradiation of the graphene quantum dots obtained from the electrochemical method. Those dots are essentially graphitic nanoparticles, which share optical properties similar to those of the precursor carbon nanoparticles for carbon dots. However, the surface passivation of the carbon nanoparticles in carbon dots (or similarly in surface-passivated graphene quantum dots) substantially improves the optical properties, as is often reflected in the much enhanced fluorescence emissions. Because the emissive excited states are likely responsible for the observed bactericidal functions, carbon dots with the more effective surface passivation and correspondingly more fluorescence are likely more desirable in serving as visible-light-activated bactericidal agents for a variety of bacteria control applications. In further investigations, steady-state and kinetic studies that correlate the optical properties of carbon dots, such as different fluorescence quantum yields and average lifetimes at various emission colors, with their antibacterial performance will be pursued.

### CONCLUSION

The results obtained in this work, while somewhat more qualitative than quantitative in some of the experiments, demonstrate unambiguously that carbon dots can readily be activated by visible light (or even under ambient room lighting conditions) for significant bactericidal functions. The light sensitivity of carbon dots is apparently rather high to the degree that would require unusually stringent experimental conditions for the dark control, an issue (such as that in Figure 5) to be examined more closely and quantitatively in further investigations, although the determination of viable cell numbers (Figure 6) should remain a more favorable method in general. Also investigated will be the issues important to the quantification of the light-activated biocidal functions, including their correlations with the properties of carbon dots in various structural and surface configurations.

### EXPERIMENTAL SECTION

**Materials.** Carbon nanopowder (purity >99%) and 2,2′-(ethylenedioxy)dibenzyldiene (EDA) were purchased from Sigma-Aldrich. Thioyl chloride (>99%) was obtained from Alfa Aesar and nitric acid from VWR. Dialysis membrane tubing of various cutoff molecular weights was supplied by Spectrum Laboratories. Water was deionized and purified by being passed through a Labconco WaterPros water purification system.

**Measurement.** Baxter Megafuge (model 2630), Eppendorf (model 5417 R), and Beckman-Coulter (Optima L90K with a type 90 Ti fixed-angle rotor) ultracentrifuges were used for centrifugation at various g values. Optical absorption spectra were recorded on a Shimadzu UB2501-PC spectrophotometer. Fluorescence spectra were measured on a Jobin-Yvon emission spectrometer equipped with a 450 W xenon excitation source, Gemini-180 excitation and Tirax-550 emission monochromators, and a Hamamatsu R928P PMT photon-
counting detector operated at 950 V. AFM images were acquired in the acoustic AC mode on a Molecular Imaging PicoPlus AFM system equipped with a multipurpose scanner and a NanoWorld point probe NCH sensor. Height profile analysis was assisted by using the SiPip software distributed by Image Metrology, TEM images were obtained on a Hitachi H9500 high-resolution TEM system.

**Carbon Dots.** For carbon nanoparticles as the precursors for carbon dots, an as-supplied carbon nanopowder sample (1 g) was refluxed in an aqueous nitric acid solution (5 M, 90 mL) for 48 h. The reaction mixture was cooled to ambient temperature and then dialyzed against fresh water for up to 3 days. The postdialysis mixture was centrifuged at 10000g to retain the supernatant, followed by the removal of water to obtain the desired carbon nanoparticle sample.

In the synthesis of EDA carbon dots using a previously reported procedure, the carbon nanoparticle sample obtained from the processing above was refluxed in neat thionyl chloride for 12 h, followed by the removal of excess thionyl chloride under nitrogen. The post-treatment carbon nanoparticle sample (50 mg) was mixed well with carefully dried EDA (500 mg) in a flask, heated to 120 °C, and stirred vigorously under nitrogen protection for 3 days. The reaction mixture was cooled to ambient temperature, dispersed in water, and then centrifuged at 20000g to retain the dark supernatant as an aqueous solution of the as-synthesized sample. The solution was dialyzed in membrane tubing (cutoff molecular weight ~500) against fresh water to remove free EDA and other impurities to obtain the EDA carbon dots in aqueous solution. For microscopy characterization, only, the EDA carbon dots were very lightly coated with gold by visible-light irradiation of the solution with HAuCl₄ for a few minutes.³¹

**Light-Activated Bactericidal Functions.** For one set of experiments, E. coli (K12) cells were cultured in fresh nutrient broth overnight, and OD₆₀₀ values of these cultures were measured and then standardized to 1 OD/mL. The bacteria suspension at 0.2 OD/mL per well was inoculated in a 12-well plate. Four treatment groups with different concentrations of carbon dots at 0.25, 0.50, 1, and 2 μM were exposed under light in a safety cabinet for T = 0, 15, 30, and 60 min, respectively. Then, the 12-well plate was incubated at 37 °C for 21 h. The OD₆₀₀ value of each well was recorded, and the readings were standardized to that of the T = 0 control plate.

For another set of experiments, fresh grown E. coli (K12) cells in nutrient broth (Fisher Scientific, Pittsburgh, PA) were washed three times and then resuspended in deionized water. With the use of 96-well plates, to a well was added a bacteria–carbon dots mixture (150 μL), in which the bacteria concentration was fixed at 1.0 × 10⁸ CFU/mL and the concentration of carbon dots was varied (triplicate for each concentration). The plates were either exposed to visible light (12 V, 36 W light bulb) or kept in the dark for 30 min. The solutions in the wells were then transferred to 1.5 mL centrifuge tubes, followed by centrifugation at 8000 rpm for 5 min. The supernatants were discarded, and the bacterial pellets were washed twice with deionized water. The cells were resuspended in 500 μL of nutrient broth, with 150 μL distributed into the wells of a 96-well plate for incubation at 37 °C. The growth of carbon-dot-treated bacterial cells and untreated cells (as controls) were monitored by measuring the OD595 values at various time points on a Spectra Max M5 spectrophotometer (Molecular Devices, LLC, Sunnyvale, CA).

For the viable cell number determination by using the traditional plating method, the suspended E. coli cells post-treatment with various concentrations of carbon dots were centrifuged and washed twice. The cells were resuspended in phosphate-buffered saline (PBS), and a series of dilutions were made with PBS. Aliquots of 100 μL appropriate dilutions were surface-plated on Luria–Bertani agar plates (Fisher Scientific, Pittsburgh, PA), and the plates were incubated at 37 °C for 24 h. The number of colonies was counted, and the viable cell numbers of the treated samples and controls were calculated in colony-forming units per milliliter.

In the use of the direct plating method for evaluation of the light-activated bactericidal functions, an E. coli (TOP10) stock culture was activated in fresh tryptic soy broth at 37 °C overnight. The bacteria culture (1 mL) was washed twice by a combination of centrifuging at 4000 rpm and resuspending in sterile PBS. The resulting cell suspension was 10-fold serially diluted in PBS. For detection of the cell concentration, aliquots of 100 μL dilutions were plated onto TSA plates and incubated at 37 °C overnight before counting. Separately, the E. coli suspension (50 μL) at a concentration of 1.3 × 10⁸ CFU/mL was mixed with aqueous solution of EDA carbon dots (50 μL) at a concentration of 1 mg/mL. The mixture was then plated onto TSA plates, which were exposed to LED light (Osram Sylvania LED A19 lamp, ~10 mW/cm²) for up to 6 h, along with the dark control (without light exposure), light control (without carbon dots), and negative control (without carbon dots and light). The carbon-dot-treated plates with light exposure and all control plates were incubated at 37 °C for 24 h before counting to determine the viable cell numbers.

All experiments were performed in triplicate or more. Statistical analysis of the experimental results was performed using the Student t test, with P < 0.05 considered a significant difference.

**REFERENCES**

Fluorescent carbon ‘quantum’ dots from thermochemical functionalization of carbon nanoparticles

Monica I. Rednic a, b, Zhuomin Lu a, c, Ping Wang a, Gregory E. LeCroy a, Fan Yang a, Yun Liu a, Haijun Qian d, Anamaria Terec b, L. Monica Veca d, Fushen Lu c, Ya-Ping Sun a, *

a Department of Chemistry and Laboratory for Emerging Materials and Technology, Clemson University, Clemson, SC 29634, USA
b Faculty of Chemistry and Chemical Engineering, Babes-Bolyai University, Cluj-Napoca 400084, Romania
c Department of Chemistry, Shantou University, Shantou, Guangdong 515063, China
d National Institute for Research and Development in Microtechnologies, IMT-Bucharest, Bucharest 077190, Romania

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A B S T R A C T

Fluorescent carbon ‘quantum’ dots are generally obtained by deliberate chemical functionalization of carbon nanoparticles or by ‘one-pot’ carbonization processing. For brightly fluorescent carbon dots with optoelectronic polymers, a hybrid approach was developed to use pre-processed and selected carbon nanoparticles as precursor for surface passivation by poly(9-vinylcarbazole) (PVK) in one-pot thermochemical processing, thus taking advantage of the more controllable feature from the deliberate functionalization and also the versatility associated with the one-pot synthesis. These PVK-carbon dots were characterized by optical spectroscopy, microscopy, and other techniques. The broad applicability of the hybrid approach is discussed.

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1. Introduction

Carbon ‘quantum’ dots or more appropriately called carbon dots for a lack of the classical quantum confinement effect in these nanomaterials were found and have since been developed as a competitive alternative to conventional semiconductor quantum dots (QDs) [1–4]. Generally carbon dots are defined as small carbon nanoparticles with various surface passivation via modification or functionalization [1–4]. Ever since the inception [1], these new fluorescent carbon nanomaterials have been investigated extensively, from the development of synthetic strategies to structural and mechanistic understandings and to the exploration on a variety of potential applications [2–4]. The effort on using carbon dots in optoelectronics as an example, Wang et al. synthesized non-aqueous carbon dots with hexadecylamine as the surface passivation agent for their electroluminescence in light emitting diodes (LEDs) [5]. Similar approach and dot samples were employed in the fabrication of LEDs with switchable emission colors [6]. Guo et al. also pursued the preparation of carbon dots that are fluorescent at different colors with poly(styrene-co-glycidylmethylacylate) particles as precursor for processing under various selected conditions and used the resulting carbon dots in the emissive layer of LEDs [7]. In more recent studies, carbon dots from different syntheses were demonstrated as being amenable to uses in white-light LEDs [8–12]. These light emitting devices are generally designed such that the emissive layer containing the carbon dots is sandwiched by other layered components for charge injection and transport purposes, such as the optically transparent and electrically conductive PEDOT:PSS layer found in all of these devices [5–9]. Polymers such as the wide-bandgap poly(9-vinylcarbazole) (PVK) are often used for hole transport, enabling more favorable energy transfer to the electroluminescent materials like carbon dots in the devices [9,13,14].

Among more popular syntheses of carbon dots, there have been the approach of deliberate chemical functionalization of small carbon nanoparticles and the method of ‘one-pot’ carbonization processing of organic or other precursors [2–4]. The former has been more controllable, yielding carbon dots of high fluorescence quantum yields (more than 50% in some configurations) [15], but the synthesis is more tedious with some limitation in the selection of functionalization molecules or species. The latter is more versatile with respect to the introduction of desired functional groups on the resulting dot surface, but less controllable, among other processing issues [16–20]. For carbon dots in optoelectronic and related applications, their improved coupling or compatibility with the polymeric materials commonly employed in various devices may play a significant role for enhanced performance. In this work on preparing brightly fluorescent carbon dots with

* Corresponding author.
E-mail address: syapings@clemson.edu (Y.-P. Sun).

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polymers relevant to optoelectronic uses [9–14], we developed a hybrid approach to use pre-processed and selected small carbon nanoparticles as the precursor for surface passivation by PVK in one-pot thermochemical processing into carbon dots, thus to take advantage of the more controllable feature from the deliberate functionalization approach and also the versatility associated with the one-pot thermochemical synthesis. According to results from optical spectroscopy, microscopy, and other characterizations, the structural and fluorescence properties of the samples thus prepared were typical of carbon dots, except for some special features (Figure 1). The broad applicability of the hybrid approach in the synthesis of carbon dots with specifically targeted surface functional moieties is discussed.

2. Experimental

2.1. Materials

The carbon nanopowder sample (<50 nm in particle size, carbon purity 99+%) was purchased from Sigma-Aldrich, and poly(N-vinylcarbazole) (PVK, Mn ~ 90 000) from Acros Organics. Nitric acid (70%), N,N-dimethylformamide (DMF), tetrahydrofuran (THF), chloroform, chlorobenzene, and 1,2-dichlorobenzene were obtained from VWR, and deuterated chloroform for NMR measurements from Cambridge Isotope Laboratories. Dialysis membrane tubing (cutoff molecular weight ~ 500) was supplied by Spectrum Laboratories. Water was deionized and purified by being passed through a Labconco WaterPros water purification system.

2.2. Measurements

Optical absorption spectra were recorded on a Shimadzu UV2501-PC spectrophotometer. Fluorescence spectra were measured on a Jobin-Yvon emission spectrometer equipped with a 450W xenon source, Gemini-180 excitation and Tirax-550 emission monochromators, and a photon counting detector (Hamamatsu R928P PMT at 950 V). The correction for nonlinear instrumental response of the spectrometer was accomplished by using separately determined correction factors with respect to different emission wavelengths. NMR measurements were performed on a Bruker Avance 500 NMR spectrometer. AFM images were acquired in the acoustic AC mode on a Molecular Imaging PicoPlus AFM system equipped with a multipurpose scanner and a NanoWorld point probe NCH sensor. The height profile analysis was assisted by using the SjPIP software distributed by Image Metrology.

SEM imaging was carried out in the SE mode on a Hitachi HD-2000 S-TEM system, and TEM imaging on a Hitachi H-9500 TEM system, coupled with the use of carbon- or holey carbon-coated copper grids. For TEM specimen prepared via microtoming, a solid sample was embedded in acrylic resin, followed by the use of a Reichert–Jung Ultracut E microtome with a 45° angle diamond knife at room temperature to obtain thin slices of less than 100 nm in thickness.

2.3. Carbon nanoparticles and carbon dots

The as-supplied nanopowder sample was refluxed in aqueous nitric acid (2.6 M) for 24 h. Upon being cooled to ambient, the acidic suspension was centrifuged at 3500 × g to collect the supernatant, which was then neutralized with sodium carbonate. The suspension was dialyzed in a membrane tubing (cutoff molecular weight ~ 500) against fresh deionized water for 3 days, followed by vigorous centrifugation at 20 000 × g to obtain a stable dispersion of the carbon nanoparticles.

The PVK-carbon dots were prepared by thermochemical processing with microwave irradiation. The reactor was a small container (about 8 cm in diameter and 2.5 cm in height) filled with silicon carbide powder as solid bath in a commercial microwave oven. In a typical experiment, the carbon nanoparticles (20 mg) were mixed well with PVK (1 g) in a vial, and with the vial immersed in the pre-heated (microwave irradiation at 500 W for 3 min) silicon carbide bath, the solid-state mixture was irradiated in the microwave oven at 300 W for 25 min. The reacted sample cooled to room temperature under ambient conditions was dispersed in THF, and the resulting dispersion was centrifuged at 20 000 × g for 60 min to collect the supernatant as a solution of the PVK-carbon dots.

2.4. PVK/carbon dots composite films

In a typical fabrication experiment, solutions of the PVK-carbon dots and neat PVK in the solvent mixture chlorobenzene/chloroform (3/1, v/v) were prepared separately and then mixed well, followed by a gradual evaporation of the solvent until the mixture becoming viscous. The viscous solution was drop-cast onto a pre-cleaned glass substrate. A polymer film was formed after slow solvent evaporation under ambient conditions for 12 h, and the film could readily be peeled off the glass slide to be free standing (thickness on the order of 5 μm).

3. Results and discussion

The commercially supplied carbon nanopowder sample was wet-processed in procedures involving the refluxing in nitric acid solution and various combinations of dispersion and vigorous centrifugation for the purpose of harvesting small carbon nanoparticles [21,22]. The nanoparticles thus obtained were generally on the order of 10 nm or less, as reported previously with the same processing and also confirmed by results from microscopy analyses (Figure 2).
The carbon nanoparticles were mixed well with commercially supplied PVK to obtain a solid blend for subsequent thermochemical processing. The one-pot processing was accomplished with microwave irradiation [23–30], in which the preferential absorption of microwave energy by the carbon nanoparticles was expected, which likely resulted in the thermochemical functionalization of the nanoparticles by the surrounding PVK moieties. After the microwave processing, the PVK-functionalized carbon nanoparticles were dispersed in THF, followed by vigorous centrifugation to yield the soluble fraction designated as the carbon dots of PVK as the surface passivation agent (PVK-carbon dots). A solution of PVK-carbon dots appeared brightly colored (Figure 3), in contrast to the colorless appearance of neat PVK solution. The color must be associated predominantly with the core carbon nanoparticles in the carbon dots sample, with the observed broad optical absorption spectrum characteristic of π–plasmon transitions in nanoscale carbon particles (Figure 3) [21,31].

Fluorescence emissions of the PVK-carbon dots were obviously excitation wavelength dependent, with the measured fluorescence spectra progressively red-shifted corresponding to longer excitation wavelengths, similar to those found in other carbon dots already reported in the literature [2–4,22]. PVK is known as being colorless, as confirmed by the observation that the polymer sample used in this work had the absorption edge well into the UV spectral region. Therefore, the fluorescence emissions corresponding to visible excitations, such as 500 nm (Figure 3), could not be associated with PVK moieties. In terms of the presently adopted mechanistic framework on carbon dots [31], the observed bright and colorful fluorescence emissions must be from the PVK-passivated carbon nanoparticle surface (Figure 1).

The fluorescence quantum yields of the PVK-carbon dots in THF solution were measured in reference to 9,10-bis(phenylethynyl)anthracene as a standard, which has a quantum yield of unity as determined by calibration against the quinine sulfate standard [32,33]. At 440 nm excitation, the fluorescence quantum yield of the solution was found to be around 13%, which is comparable to results for other more extensively studied carbon dots from the deliberate functionalization synthetic's, such as those with the oligomeric poly(ethylene glycol) diamine (PEG1500N) as the surface passivation agent (corresponding fluorescence quantum yields of 10–20% for the as-synthesized samples in aqueous solution) [15,33]. The quantum yields of the PVK-carbon dots were lower at longer excitation wavelengths, also similar to what were observed in carbon dots of other surface functional groups [15].

Morphologically, the PVK-carbon dots are not discrete individual dots (namely that the carbon nanoparticles would each be functionalized by one or more PVK molecules), rather composite-like with the functionalized carbon nanoparticles embedded in PVK polymer networks (Figure 1), consistent with the results from scanning electron microscopy (SEM) and transmission electron microscopy (TEM) characterizations. Experimentally, the specimen for microscopy analyses was prepared by dropping a THF solution of the PVK-carbon dots onto a carbon-coated copper grid, followed by the evaporation of the solvent. As shown in Figure 4, the SEM images (obtained in the SE mode on a scanning-transmission

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**Figure 2.** Representative AFM images of the carbon nanoparticles as precursor for carbon dots on mica substrate.

**Figure 3.** Absorption (ABS) and fluorescence (FLSC, 500 nm excitation, corrected for nonlinear instrumental response of the spectrometer) spectra of the PVK-carbon dots in THF solution (---), with the absorption spectrum of aqueous suspended precursor carbon nanoparticles (----) also shown for comparison. Inset: photograph for a typical THF solution of the PVK-carbon dots.

**Figure 4.** SEM images (upper) and TEM images at low (lower left) and high (lower right) resolution for a specimen of the PVK-carbon dots.
electron microscopy or S-TEM instrument) are dominated by large particle-like features commonly found in the imaging of polymeric nanocomposites. The same specimen was analyzed by TEM at different resolutions, with the results also shown in Figure 4. The lower magnification images are similar to those from SEM, while those at high resolution suggest the embedding of carbon nanoparticles, again morphologically similar to what are typically observed in the TEM imaging of polymeric nanoparticles.

For a more detailed TEM characterization, a specimen of the PVK-carbon dots was prepared by dispersing the sample into acrylic resin for being micromed into thin slices of less than 100 nm in thickness. As shown in Figure 5, carbon nanoparticles in the PVK-carbon dots are more clearly identified in the TEM images of the specimen prepared via micromedtoming, further confirming the composite-like structural feature (Figure 1).

The PVK-carbon dots in a solution of deuterated solvent were characterized in NMR measurements, with the results compared with those of the corresponding neat PVK. As shown in Figure 6, the solution-phase $^1$H NMR signals in the spectrum of the PVK-carbon dots are similar to those of the neat PVK, without any fundamental changes in terms of their chemical shifts or the broadness in the signals. Within the proposed structural framework (Figure 1) such that the carbon dots (carbon nanoparticles functionalized by PVK polymers for the observed bright fluorescence emissions in the visible spectral region) are composites with the polymers (Figures 4 and 5), the NMR results suggest that the presence of carbon dot domains in the overall composite-like sample structures do not significantly change the already diverse environments for the PVK protons. Therefore, the primary effect of the thermochemical synthesis seems to be only the introduction of the pre-processed and selected carbon nanoparticles into the PVK network for being surface-passivated to enable bright and colorful fluorescence emissions that are essentially the same as those from the ‘conventional’ carbon dots already reported in the literature [1–4].

The already composite-like nature of the as-synthesized PVK-carbon dots samples made them ideal precursors in solution-phase processing for the fabrication of optically transparent PVK films dispersed with fluorescent carbon dots (Figure 7). In a typical fabrication protocol, a carbon dots sample was mixed well with a selected amount of neat PVK in solution, and the mixture was concentrated for wet-casting onto a clean glass slide. The PVK/carbon dots composite film thus formed could be peeled off to be free-standing (Figure 7). The film, special in terms of being free from any other materials or reagents besides the nanoscale carbon particles and PVK, exhibited largely similar optical absorption and fluorescence emissions to those of the PVK-carbon dots in solution (Figure 7).

The hybrid approach in this work that combines the more controllable feature of functionalizing pre-processed and selected carbon nanoparticles with the advantage of thermochemical synthesis enables highly versatile access to carbon dots of a variety of surface passivation agents. In comparison with the deliberate and specific chemical functionalization method, the hybrid approach is subject to much less constraints (including those that might be prohibitive), considerably more efficient, especially with the use of microwave processing. On the other hand, the use of pre-processed and selected carbon nanoparticles as precursor not only removes the limitation of relying on the direct carbonization to form size-wise homogeneously distributed carbon nanoparticles, which is generally speaking difficult or unlikely. In addition, there are abundant and inexpensive carbon sources from which the precursor carbon nanoparticles could be processed and selected for specific needs or configurations of the final carbon dots.

The carbon dots from the thermochemical functionalization of pre-processed and selected carbon nanoparticles were apparently very stable, probably due to the expected functionalization mode of PVK moieties being ‘melted’ onto the carbon nanoparticles. The samples and sample solutions of the PVK-carbon dots exhibited
similar optical properties over a significant period of time, such as being stored under ambient conditions for several months.

4. Conclusion

As demonstrated in this work, the hybrid approach is more useful to the preparation of polymer-functionalized carbon dots for strongly absorptive and fluorescent nanocomposites without any unwanted foreign materials (such as the surface passivation agents in the ‘conventional’ carbon dots), thus with improved properties and performance. This is particularly valuable to nanocomposite materials targeted for optoelectronic devices, in which the presence of any foreign materials (essentially ‘impurities’) might have significant negative effects, such as those due to potentially microscopic phase separation.

The optical properties of the carbon dots from thermochemical synthesis reported here, including the fluorescence brightness, are in line with those from the deliberate chemical functionalization method, though somewhat toward the lower end. Therefore, further investigations on the hybrid approach for significant performance improvements of the resulting carbon dots, especially for higher fluorescence quantum yields across the visible spectrum, represent both challenges and opportunities.

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References

Host-Guest Carbon Dots for Enhanced Optical Properties and Beyond

Ya-Ping Sun, Ping Wang, Zhuomin Lu, Fan Yang, Mohammed J. Meziani, Gregory E. LeCroy, Yun Liu & Haijun Qian

Carbon dots, generally small carbon nanoparticles with various forms of surface passivation, have achieved the performance level of semiconductor quantum dots in the green spectral region, but their absorption and fluorescence in red/near-IR are relatively weaker. Conceptually similar to endofullerenes, host-guest carbon dots were designed and prepared with red/near-IR dyes encapsulated as guest in the carbon nanoparticle core. Beyond the desired enhancement in optical properties, the host-guest configuration may significantly broaden the field of carbon dots.

Carbon dots (also called carbon quantum dots in some literature reports despite the absence of classically defined quantum confinement) have emerged as a new class of photoactive nanomaterials, with their fluorescence properties resembling those typically found in conventional semiconductor nanocrystals or quantum dots (QDs). The structure of a carbon dot is relatively simple, generally a small carbon nanoparticle with various forms of surface passivation, among which the more effective has been the chemical functionalization with organic or polymeric species. In the green over the spectral region covered by green fluorescent protein (GFP), for example, the performance of existing carbon dots in terms of fluorescence quantum yields in solution or the image brightness at the individual dot level on a substrate has been found to be competitive to that of the presently dominating CdSe/ZnS QDs. According to available experimental results, carbon dots are nontoxic, certainly without the toxicity concerns associated with the heavy metal-containing semiconductor QDs. Therefore, there has been a growing interest in potential applications of carbon dots for fluorescence bioimaging in vitro and in vivo. However, despite the extensive effort in the relevant research community, the development of carbon dots of high fluorescence quantum yields in the biologically more significant near-IR spectral region has found only limited success. This, combined with the generally lower absorptivity of carbon nanoparticles in the red/near-IR, suggests that new strategies are necessary in order to use the carbon dots platform for fluorescence probes of the desired red/near-IR performance.

In the work reported here we “borrowed” the concept from the field of endofullerenes by considering the core carbon nanoparticle in a carbon dot as a “solid-state pool” (versus the cavity in a fullerene) to trap or encapsulate chromophoric species of strong red/near-IR absorption and emissions. This host-guest configuration takes advantage of the small carbon nanoparticle as host being optically largely transparent in the corresponding spectral regions, which has actually been identified above as a shortcoming of currently available carbon dots in their serving as red/near-IR probes. The resulting host-guest carbon dots, denoted as G@CDots, exhibited the desired absorption and fluorescence properties, as designed and expected.
Results and Discussion

The thermal carbonization of organic precursors has been a popular approach for the synthesis of carbon dots\(^2\)–\(^4\), in which a portion of the precursor organic species is converted into carbon nanoparticles and the remaining serves the function of surface passivation agents. Among various thermal processing options is the use of microwave irradiation\(^1\)\(^4\)–\(^2\)\(^1\)\(^–\)\(^2\)\(^1\)\(^–\)\(^2\)\(^1\), which in principle creates carbonized seeds for their preferential absorption of the subsequent microwave energy towards the formation of the targeted carbon dot structure on a carbon nanoparticle with organic species on the surface for passivation. The microwave processing was adopted in this work for the “one-pot” synthesis of the G@CDots, with G denoting the selected fluorescent dyes of cresyl violet (CV), nile blue (NB), and zinc phthalocyanine (ZnPc).

Experimentally for the synthesis of CV@CDots, CV (20 mg) in an ethanol solution was mixed well with oligomeric polyethylene glycol of molecular weight \(\sim 900\) (PEG 900, 2 g), followed by the removal of ethanol via purging with nitrogen gas. The resulting mixture was placed in a commercial microwave oven and irradiated at 300 W for 20 min. Then, water was added to the reaction mixture with sonication to obtain a dark colored aqueous solution. The solution was centrifuged at 20,000 \(g\), from which only a negligible amount of precipitate was observed and discarded. The supernatant was dialyzed in a membrane tubing (cutoff molecular weight \(\sim 1,000\)) against fresh water to remove unreacted starting materials and other small molecular species, yielding CV@CDots in an aqueous solution. The same processing protocol was applied to the preparation of NB@CDots and ZnPc@CDots, except that for the latter a 1:1 mixture of PEG 900 and oligomeric polypropionylethyleneimine instead of neat PEG 900 was in the mixture with ZnPc for microwave irradiation. The sample solutions were used for optical spectroscopy measurements and microscopy characterization.

For all three host-guest carbon dots, the absorption spectra in aqueous solutions exhibited contributions from carbon nanoparticles (more significantly in the blue/green spectral region, comparable with the absorption of carbon dots from the carbonization of PEG\(_{900}\) without the dye encapsulation, Fig. 2) and the guest dye molecules (Figs 2 and 3). However, the absorption bands of the encapsulated dyes are somewhat different from those of their corresponding free molecules, likely reflecting effects of the different environment in the hosting carbon nanoparticles. For example, the absorption of the CV in CV@CDots is much broader in comparison with that of the free dye molecules, both in aqueous solutions (Fig. 2). Similar encapsulation effects were observed in fluorescence spectra of the host-guest carbon dots. The spectra were found to be excitation wavelength dependent, as shown in Fig. 2 for example, which might be as expected considering the solid-like environment around the guest dye molecules in the hosting carbon nanoparticles (namely the molecules are each in a slightly different surrounding in a “solid-state solution”, a classical case for excitation wavelength dependent fluorescence emissions). Similarly for ZnPc@CDots in aqueous solution excited at its absorption peak, the fluorescence band is broader and red-shifted from that of the free ZnPc molecules (Fig. 3).

The aqueous solutions of the host-guest carbon dots were diluted for the preparation of specimens on mica substrate for atomic force microscopy (AFM) characterization. Shown in Fig. 4 are the results for CV@CDots, NB@CDots, and ZnPc@CDots. According to image height analyses, these host-guest carbon dots synthesized from thermal carbonization reactions are not as uniform in size as those from the surface chemical functionalization of pre-processed carbon nanoparticles reported previously\(^6\)\(^,\)\(^2\)\(^2\), though still relatively narrowly distributed. Most of these host-guest carbon dots are small, with their overall size profiles on the order of 10 nm or less (Fig. 4).

The carbon nanoparticle cores in the host-guest carbon dots are likely somewhat smaller than the overall dot profiles estimated from the height analysis of AFM images, as the latter may also include contributions of the organic species on carbon particle surface that survived the thermal carbonization processing. The expected significant contrast between the carbon core and surface organic species was exploited in the probing of the carbon nanoparticles by using transmission electron microscopy (TEM). For NB@CDots as an example, the TEM specimen was prepared such that a few drops of a dilute sample solution were deposited onto a silicon oxide-coated copper grid, followed by careful evaporation of the solvent. The imaging experiments were performed on a high-resolution TEM instrument (Hitachi...
Figure 2. The absorption (ABS) spectrum of CV@CDots (—) and corresponding fluorescence (FLSC) spectra (excitation at 570 nm: —, 600 nm: -.-, and 620 nm: -.-) in aqueous solution. The spectra of free CV (— - -) and carbon dots from the carbonization of PEG\textsubscript{900} without any encapsulation (…) in aqueous solutions are also shown for comparison. Inset: Photographs of an aqueous solution of the sample under UV light in the dark (left) and under natural day light (right).

Figure 3. Absorption (ABS) and fluorescence (FLSC) spectra of NB@CDots (top, —) and ZnPc@CDots (bottom, —) and the corresponding free dyes ( - - - ) in aqueous solutions (except for free ZnPc in DMSO). Insets: Photographs of aqueous solutions of the corresponding samples under UV in the dark (left) and under natural day light (right).
The results shown in Fig. 5 suggest that the NB-encapsulated carbon dots with residual PEG molecules as surface passivation moieties (confirmed by the significant PEG carbon peaks in $^{13}$C NMR analyses) are well-dispersed and that the carbon nanoparticle cores are size-wise small and relatively narrowly distributed.

For the host-guest carbon dots in aqueous solutions, the fluorescence quantum yields of the encapsulated dyes were evaluated against those of their free counterparts. Mechanistically, the observed fluorescence emissions from the guest dyes were due to their intrinsic electronic transition properties, not induced by the host carbon dots. However, the carbon pool environment in the hosting carbon dots could have meaningful effects on the fluorescence properties of the guest dyes. Among the three selected dyes, CV is soluble in water$^{23}$, NB less so and only weakly fluorescent in an aqueous environment$^{24}$, and ZnPc soluble in organic solvents$^{23}$. Generally the results suggested that the fluorescence quantum yields of CV and NB as guests in the host-guest carbon dots were similar to those of free CV and NB molecules, respectively, all in aqueous solutions. More specifically for CV, it is known in the literature that

Figure 4. AFM images of CV@CDots (top), NB@CDots (middle), and ZnPc@CDots (bottom).
its fluorescence quantum yields in aqueous solutions are somewhat concentration dependent, higher in a more dilute solution, yet overall about 40% lower than the yields in methanol. The observed similar fluorescence quantum yields between the encapsulated and free CV molecules might be due to the opposing effects of a relatively higher CV concentration and more non-aqueous environment in CV@CDots, which decreases and enhances the quantum yields, respectively. However, for NB@CDots in an aqueous solution, the estimated fluorescence quantum yields of the guest NB were higher than that of free NB molecules in water (on the order of 0.01) but still significantly lower than that in ethanol (around 0.27), probably suggesting that the environment for the encapsulated NB is not entirely free from water. Similarly, the fluorescence quantum yields of ZnPc as guest in the host-guest carbon dots in an aqueous solution were also significantly lower than those of free ZnPc molecules in organic solvents, likely also due to the exposure of the encapsulated ZnPc to water (because the ZnPc fluorescence in a polar organic solvent is apparently quenched efficiently by the addition of water). Therefore, in further investigations the fluorescence properties of these water-sensitive dyes may be used to study the local environment in the core carbon nanoparticles in the host-guest carbon dots. Experimentally, more effort is needed to correct the light scattering effect in aqueous solutions of the host-guest carbon dots for a more accurate determination of the fluorescence quantum yields of the encapsulated dyes.

Conceptually similar to endofullerenes that have expanded the horizons of the fullerene field, the host-guest carbon dots represent a new QD-like nanoarchitecture for materials properties and functions beyond those achieved with the original carbon dots. The extension of absorption and fluorescence coverage of carbon dots into the red/near-IR spectral regions with the relevant dyes as guest in this work serves as a representative example for the potential and versatile nature of the host-guest carbon dots platform. Such a new platform is expected to significantly broaden the reach of the already rapidly advancing carbon dots research and development.

References


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Author Contributions
Y.-P.S. came up with the idea and supervised the performance of the work; P.W., Z.L. and F.Y. performed various tasks of the work; M.J.M. and H.Q. contributed to the sample analyses; and G.E.L. and Y.L. assisted the performance of the work.

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Carbon nanotube-assisted capturing of bacterial pathogens

Shengyuan Wang, Gregory E. LeCroy, Fan Yang, Xiuli Dong, Ya-Ping Sun, and Liju Yang

This study explored the use of co-polymer poly(propionylethyleneimine-co-ethyleneimine) (PPEI-EI) functionalized multi-walled carbon nanotubes (MWNTs) as a coating material on filters for capturing of bacterial pathogens from aqueous solutions. Polycarbonate membranes with pore sizes of 1.2 and 3.0 μm were coated with different PPEI-EI-MWNTs and cross-linked PPEI-EI-MWNTs samples at various coating densities, and then evaluated for capturing of E. coli cells at flow rates of 0.25 and 0.5 mL min⁻¹. With a good combination of PPEI-EI-MWNTs sample, coating density, appropriate filter pore size and flow rate, a capture efficiency of higher than 4 log (up to 6 log or larger) of bacterial cells was achieved. The filters coated with the cross-linked PPEI-EI-MWNTs were unexpectedly less efficient than those with the other PPEI-EI-MWNTs samples, likely due to the poorer dispersibility of the cross-linked sample and consequently the less homogeneous coating on filters. The results of this study demonstrated the feasibility of PPEI-EI-MWNTs as a coating material on filters for highly effective capturing of bacterial pathogens, and also presented both challenges and opportunities for further investigations in controlling the coating material synthesis to improve performance in capturing bacterial cells.

Introduction

Foodborne illness is recognized as a common and costly public health problem around the world. The Centers for Disease Control and Prevention (CDC) estimates that, in the United States alone, each year, foodborne outbreaks cause sickness in 48 million people, accounting for 128,000 hospitalizations and 3000 deaths. A variety of foodborne pathogens, including bacteria, viruses, and parasites, have been known for causing more than 250 different foodborne diseases. However, these foodborne diseases are preventable through effective strategies for pathogen detection, isolation/removal, or inactivation. A major challenge for both preventing and responding to the contamination of pathogens is that the numbers of the pathogens in most food and related samples are relatively low, so that their isolation and concentration are necessary to enable rapid detection and analyses. In the technological development for such a purpose, there have been some significant advances in new or improved methods for capturing and concentrating pathogens, such as microfluidic filtration, nanoparticle-based immuno-separation, and isoelectric focusing. Membrane-based separation represents a class of methods that can be incorporated into a range of devices for the isolation or concentrating of pathogens for subsequent detection. In practice, these methods are still challenged in dealing with multiple pathogens in various matrices in addition to other technical issues, therefore new approaches for effective yet low-cost methods are in demand.

The development of nanotechnology and its successful integration with biotechnology offer excellent opportunities in addressing the pathogen isolation or concentrating issues. In this regard, carbon nanotubes (CNTs) have been shown as being particularly promising. CNTs are well-ordered, hollow carbon fibers with a high aspect ratio [lengths from several hundred nanometers to several micrometers; diameters of 0.4–2 nm for single-walled carbon nanotubes (SWNTs), and 2–30 nm for coaxial multiple-walled carbon nanotubes (MWNTs)]. CNTs possess remarkable properties that have been explored for a wide range of biological applications. Particularly, research results obtained in our laboratories and by others have demonstrated two major promising properties of CNTs that are applicable in the microbiology field: the high absorptivity for removing biological contaminants and the strong antimicrobial activity for inactivation of bacterial cells. These properties have afforded CNTs the potential to make important advancements in dealing with foodborne bacterial pathogens. Especially, their microbial absorption capacities are proven to be higher than any other commercially available adsorbent media.

CNTs are insoluble, which makes their dispersion and processing a major challenge. The chemical modification or
functionalization has become an effective strategy in the solubilization and dispersion of CNTs for various purposes.\textsuperscript{11,18,19} For example, it has been demonstrated that CNTs could be functionalized covalently or non-covalently with a series of biologically significant oligomers or polymers,\textsuperscript{11,20–22} including poly(ethylene glycol) (or PEG), proteins (bovine serum albumin or BSA) for further conjugation with antibody\textsuperscript{23–25} to target specific pathogens,\textsuperscript{25,26} and various carbohydrates for cell adhesion and related applications.\textsuperscript{23–25,27–29} The surface functional moieties coupled with the intrinsic high absorptivity of CNTs have found these functionalized nanotube samples in applications such as the removal, concentration and/or detection of pathogens.\textsuperscript{23,25}

In the work reported here, we designed and synthesized several configurations of polymer functionalized MWNTs, and used them in the coating of filters for their capturing of bacterial cells. We used an inexpensive co-polymer poly(propionylethenimine-co-ethylenimine) (PPEI-EI) for the nanotube functionalization. The variations in the functionalized MWNT samples included PPEI-EI of two different molecular weights (5000 and 50 000), different functionalization chemistries and processing conditions (amidation and thermochemical methods), and different post-functionalization sample selections (gravimetric fractionation and cross-linking to yield samples containing networked species for more filter-coating options). We evaluated the efficiencies of filters coated with different PPEI-EI-MWNTs samples and also cross-linked PPEI-EI-MWNTs samples for capturing of Escherichia coli K12 cells, from which the results confirmed the feasibility of using polymer-functionalized CNTs in the development of rapid, low-cost, and efficient methods for capturing/isolation of pathogens.

**Experimental**

**Materials**

The multiple-walled carbon nanotube (MWNT, 95% purity, 10–20 nm in diameter and ~15 µm in average length) sample was supplied by Nanostructured and Amorphous Materials, Inc, and poly(2-ethyl-2-oxazoline) (PPEI, average $M_w$ ~ 5000 and 50 000) from Polysciences, Inc. Nitric acid (70%) and chloroform (99.9%) were purchased from Fisher Scientific, hydrochloric acid (38%) from EMD, sodium hydroxide (98%) from Sigma Aldrich, thionyl chloride (99%) from Alfa Aesar, and methanol (99.8%) from Mallinckrodt Chemicals. Deuterated chloroform with TMS internal standard for NMR measurements was acquired from Cambridge Isotope Laboratories. Dialysis membrane tubing was obtained from Spectrum Laboratories. Water was deionized and purified by being passed through a Labconco WaterPros water purification system.

**Measurements**

Desktop centrifuges (Fisher Scientific Model 228 and Eppendorf Centrifuge 5417R) and sonicator (VWR Model 250D) were used in purification and functionalization experiments. Thermal gravimetric analysis (TGA) was performed on a TA Instruments SDT-Q600 system. NMR measurements were carried out on a Bruker Avance 500 spectrometer. Scanning electron microscopic (SEM) images were taken using the FEI XL30 microscope (Netherlands) at the Shared Materials and Instrumentations Facility (SMIF) at Duke University. Uncoated filters and PPEI-EI-MWNTs coated filters with and without bacterial filtration were examined under SEM to determine the distribution of PPEI-EI-MWNTs on the filters and the interactions between bacterial cells and the coated filters. For filters with bacterial cells, the cells were first fixed in bacterial fixative (4% formaldehyde and 2% glutaraldehyde in 1 × PBS) for 6 h at room temperature. After washing with DI water, the samples were air dried. Filters without bacterial cells did not need the fixing step. Before SEM imaging, all filters were coated with gold using Denton Vacuum Desk IV (Czech Republic) as previously described.\textsuperscript{26}

**PPEI-EI-MWNT**

The as-supplied MWNT sample was purified to remove carbon impurities and catalysts by following a protocol reported previously.\textsuperscript{27} Briefly, the sample (1 g) was refluxed in aqueous nitric acid (2.6 M, 200 mL) for 48 h, followed by centrifugation and washing repeatedly with deionized water to retain the residue and then drying under vacuum. The PPEI polymer with $M_w$ of either 5000 or 50 000 was partially hydrolyzed to obtain random copolymer poly(propionylethenimine-co-ethylenimine) (PPEI-EI).\textsuperscript{27} In a typical reaction, PPEI (5 g) was dissolved in methanol (15 mL), and the solution was transferred to deionized water (250 mL), followed by evaporation to remove methanol. To the resulting aqueous dispersion of the polymer was added aqueous HCl (1 M, 2 mL), and the acidified dispersion was refluxed with stirring for 12 h. The reaction mixture was cooled to ambient, and then aqueous NaOH (1 M) was added to adjust pH to ~10. The mixture was concentrated by removing water and extracted with chloroform to obtain PPEI-EI. The copolymer was further purified by repeated precipitation from the concentrated chloroform solution into hexanes. Upon the removal of solvents and drying in vacuum at 50 °C, PPEI-EI was recovered as a glassy solid sample. \textsuperscript{1}H NMR (500 MHz, CDCl$_3$) δ 3.45 (broad, NCH$_2$), 2.78 (broad, NHCH$_{3}$), 2.4 (broad, COCH$_2$CH$_3$), 1.12 (broad, COCH$_2$CH$_3$) ppm. The NMR signal integrations were used to estimate the EI fraction in PPEI-EI, which was generally 15–20% among different precursor PPEI samples and different batches.

MWNTs were functionalized with PPEI-EI under classical amidation reaction conditions.\textsuperscript{27,28} In a typical experiment, the purified MWNT sample (50 mg) was acylated by refluxing in neat thionyl chloride for 12 h. Upon removal of excess thionyl chloride, the acylated nanotubes were mixed with carefully dried PPEI-EI (500 mg), heated to 200 °C, and vigorously stirred under nitrogen for 72 h. The reaction mixture was cooled to ambient, and extracted with water for the PPEI-EI-functionalized MWNTs (PPEI-EI-MWNT) in an aqueous dispersion. Cross-linking of the functionalized nanotubes was performed by hydrothermal processing in a sealed reactor.
PPEI-EI-MCNT coated filters

In the coating of commercially supplied membrane filters, a selected PPEI-EI-MCNT sample in aqueous dispersion was diluted with deionized water to the desired concentration. Aliquots of 450 μL of the aqueous dispersed nanotube sample at different concentrations were deposited on either 3.0 μm pore sized TSTP hydrophilic polycarbonate membranes or 1.2 μm pore sized RTTP hydrophilic polycarbonate membranes (both membranes of a diameter of 25 mm) (EMD Millipore, Billerica, MA) to attain a PPEI-EI-MWNT loading ranging from 0.095 μg mm⁻² to 1.25 μg mm⁻² on the base filter. The filter membranes were held in a filter holder (EMD Millipore, Billerica, MA), and were dried in a fume hood overnight.

Bacteria culture

Escherichia coli K12 was grown in 10 mL nutrient broth by inoculating the broth with a single colony of a plated culture on a Luria–Bertani (LB) agar plate, and incubated overnight at 37 °C to reach 10⁸ CFU mL⁻¹ to 10⁹ CFU mL⁻¹. The actual bacterial cell concentration was determined by the traditional surface plating of appropriate serial dilutions on LB agar plates. The plates were incubated at 37 °C overnight, and the colonies were counted to calculate the bacterial cell concentration in the original culture as colony forming unit per milliliter (CFU mL⁻¹). For the use in further filtration experiments, E. coli cells were diluted with DI water to a concentration of ~2.5 × 10⁶ CFU mL⁻¹.

Bacterial sample filtration

Aliquots of 2.5 mL E. coli suspension in DI water were filtered by uncoated and PPEI-EI-MWNT coated filters through a syringe and a syringe pump (Harvard Apparatus PHD 2000 Infuse/Withdraw) with flow rate at 0.5 mL min⁻¹ or 0.25 mL min⁻¹. Filtrates were collected in centrifuge tubes, and E. coli concentrations in the filtrates were determined by the traditional surface plating with the same procedure described above. The reduction in logarithmic value of E. coli cell number before and filtration was used to determine the efficiency of various coated filters for bacterial capture.

Statistical analysis

Statistical analysis was performed using the Student’s t-test. Differences were considered to be significant at the level of P < 0.05.

Results and discussion

MWNTs are in general difficult to be dispersed in water or other solvents, which limits their further processing or being incorporated into various devices such as the coated filters targeted in this study. A common approach for much improved dispersibility has been the functionalization of MWNTs with oligomeric and polymeric species. The aminopolymer poly(propionylethylenimine-co-ethylenimine) (PPEI-EI, Scheme 1) was used for the functionalization. It is well-known that MWNTs purified in processing with oxidative acid such as nitric acid contain surface sites that are populated with carboxylic acid moieties. These were targeted for the attachment of PPEI-EI in functionalization under classical amidation reaction conditions, where the EI moieties in PPEI-EI copolymers (Scheme 1) were used to form amide linkages to the nanotubes. Beyond the desired dispersibility, the polymer functionalization could also improve the adhesion of the nanotubes to the membrane filters to be used for capturing bacterial cells in the filtration of the cell solution, and/or possibly influence the capture of bacterial cells by adjusting the molecular weight of the polymer. PPEI-EI copolymers of average molecular weight 5000 and 50 000 were used for the amidation reactions, yielding their functionalized MWNT samples denoted as PPEI-EI(5000)-MWNT and PPEI-EI(50 000)-MWNT, respectively.

The functionalized MWNTs were obtained in aqueous dispersions. The nanotube concentrations in the dispersions were determined by taking out accurately measured aliquots for the removal of water, drying, and then thermal gravimetric analysis (TGA). The TGA under inert gas purging allowed the selective removal of PPEI-EI, thus the determination of nanotube contents in the samples. The concentrations used in this study are referred to the nanotube contents determined by TGA.

Commercially acquired hydrophilic polycarbonate filters were coated with the PPEI-EI-functionalized MWNTs, for which the protocol to evaluate the efficiency in capturing bacterial cells was based on the enumeration of viable cell number in the filtrate using the traditional surface plating method, with the results compared to those in unfiltered samples and/or those in filtrate using uncoated filters.

We first examined the effect of molecular weight of PPEI-EI on the bacterial capture efficiency by using PPEI-EI(5000)-MWNT and PPEI-EI(50 000)-MWNT samples to coat filters (3.0 μm pore size) for the purpose of capturing bacterial cells from solutions. Fig. 1 shows the efficiency of the coated filters for capturing of E. coli cells. The uncoated filters were unable to capture E. coli cells from solutions, as the cells were easily passing through the pores on the filters. The coating with PPEI-EI-MWNT enabled the filters to capture E. coli cells. The results indicated that the filters coated with PPEI-EI(5000)-MWNT were able to capture approximately 1.79 log of cells from a solution containing 1.88 × 10⁶ CFU mL⁻¹ E. coli cells, whereas those with PPEI-EI(50 000)-MWNT were somewhat less efficient, capturing approximately 0.91 log of cells. The ability of the coated filters to capture bacterial cells may be attributed to a combination of bacterial cells being physically blocked from

![Scheme 1] Chemical structure of PPEI-EI.
passing through the pores and the known absorptivity of CNTs to bacterial cells.\textsuperscript{39,40} The same combination of effects might also be responsible for the observed different efficiencies between filters coated with PPEI-EI(5000)-MWNT and PPEI-EI(5000)-MWNT in capturing \textit{E. coli} cells.

The pore sizes (3.0 µm) of the filters used in the experiments above were much larger than the average dimension of the \textit{E. coli} cells, so that the observed retention of the cells on the coated filters must be due to the presence of the nanotube coating. The functionalized MWNTs were of a large aspect ratio, but the overall size profiles were not significantly larger than the filter pore sizes. It was assumed that a more desirable configuration for capturing bacterial cells might be for the functionalized MWNTs to form a network on top of the pores to reduce the effective pore sizes for the passing cells during the filtration and to facilitate more effective nanotube–cell interactions.

Therefore, the cross-linked PPEI-EI-MWNT samples (designed for such a purpose and prepared with a cross-linking protocol after the functionalization reaction) were used to coat the filters (3.0 µm) at the same density. These filters were evaluated by using the same experimental protocol in their capturing \textit{E. coli} cells, and the results suggested significant capturing efficiency, with the best of 2.1 log (shown in Fig. 1), and down to 1.1 log, but still not meaningfully better than the filters coated with the nanotube samples without cross-linking.

SEM examination of PPEI-EI(5000)-MWNT, PPEI-EI(50 000)-MWNT found that the two nanotube samples appeared morphologically similar (Fig. 2A and B). However, the size profiles for individual functionalized carbon nanotubes in the two samples might still be different, and the different PPEI-EI molecular weights could also affect the way in their wrapping MWNTs and consequently the availability and nanoscale details of the exposed areas on the nanotubes (not wrapped by PPEI-EI), thus altering the absorptivity to bacterial cells, resulting in different efficiency for capturing bacteria on those coated filters. Fig. 2C shows a representative SEM image of a cross-linked sample, where less individual nanotubes and more conjugates with polymers can be seen. It is true that the cross-linked samples had poorer dispersibility, which made the coating more difficult and resulted in coated filters on which the distribution of the nanotubes was less homogeneous. And this is the likely cause of unimproved capture efficiency of filters coated with cross-linked samples.

Based on the above results, filters coated with PPEI-EI(5000)-MWNTs and the cross-linked samples showed better capture efficiencies. Next, we examined the effect of reduced pore size of the filters on the coating for capturing of bacterial cells. Filters of a smaller pore size (1.2 µm) were coated with the PPEI-EI(5000) MWNT samples and the cross-linked samples, and the coated filters were examined for their capturing bacterial cells by filtration of \textit{E. coli} suspensions containing $10^6$ to $10^7$ CFU mL$^{-1}$ at a flow rate of 0.5 mL min$^{-1}$. Fig. 3 shows the efficiencies of capturing \textit{E. coli} cells with these filters, along with the uncoated filters and the unfiltered suspensions as

![Image](https://example.com/image1)

**Fig. 1** The capture efficiencies of PPEI-EI(5000)-MWNTs, PPEI-EI(50 000)-MWNTs, and cross-linked PPEI-EI-MWNTs coated filters for the filtration of \textit{E. coli} cells from aqueous solutions containing ~$10^6$ CFU mL$^{-1}$ cells. Filters: polycarbonate. $d = 25$ mm, pore size 3.0 µm; both PPEI-EI-MWNT samples were coated on the filters at the same density of 1.01 µg mm$^{-2}$; flow rate: 0.5 mL min$^{-1}$. Statistical analysis was performed among all the samples, different letters on the columns indicate a statistical difference ($P < 0.05$), while the same letters indicate no statistical difference ($P > 0.05$).

![Image](https://example.com/image2)

**Fig. 2** SEM images of PPEI-EI co-polymer functionalized MWNTs, (A) PPEI-EI(5000)-MWNTs, (B) PPEI-EI(50 000)-MWNTs, and (C) cross-linked PPEI-EI-MWNTs.

![Image](https://example.com/image3)

**Fig. 3** The capture efficiencies of PPEI-EI-MWNTs and cross linked PPEI-EI-MWNTs coated filters for capturing of \textit{E. coli} cells from aqueous solution containing ~$10^6$ CFU mL$^{-1}$ cells. Filters: polycarbonate. $d = 25$ mm, pore size 1.2 µm; all PPEI-EI-MWNT samples were coated on the filters at the same density of 0.75 µg mm$^{-2}$. Statistical analysis was performed among all the samples, different letters on the columns indicate a statistical difference ($P < 0.05$), while the same letters indicate no statistical difference ($P > 0.05$).
controls. The uncoated filters were generally incapable of capturing bacterial cells, as the cell number in the filtrate was not significantly different from that in the unfiltered suspensions. Obviously, all PPEI-EI-MWNT-coated filters were able to retain a significant number of *E. coli* cells in general. The capturing efficiency was up to 4.66 log with a well-coated filter (Fig. 3).

However, the performance of the coated filters varied with the functionalized nanotube samples synthesized in different batches, which might be associated with slightly different material properties, especially in terms of solubility or dispersibility of the samples. These properties could affect the distribution of the nanotubes on the coated filters, resulting in somewhat different efficiencies in capturing bacterial cells, down to 2.73 log in the worst case, which were still significantly better than the coated filters of 3.0 μm pores. The results from the use of filters coated with the cross-linked sample were consistent with such an assessment, with the observed capturing efficiency up to 2.29 log as shown in Fig. 3, and down to 1.1 log in the worst case.

Since the cross-linked samples were more difficult to disperse, making the coating on the filters less homogeneous, as discussed above, the reduced pore size did not improve the coating homogeneity and the capturing efficiency. The microscopy imaging results were consistent with such an explanation as quite amount of large aggregates of cross-linked nanotubes were presented on the coated filter (Fig. 4A). The filters coated with PPEI-EI(5000)-MWNT were also examined directly under SEM, and representative results are shown in Fig. 4. On the PPEI-EI(5000) coated filters (Fig. 4B), the distribution of the functionalized MWNTs was not even, with small aggregates of the nanotubes found in some parts of the filter, and some pores, especially those adjacent pores, left uncovered in other parts of the filter. The uncoated hydrophilic polycarbonate filters clearly showed that the pores were distributed unevenly with some of them adjacent to each other, resulting in larger actual pore size (Fig. 4C). These observations present both challenges and opportunities for further investigations. The challenges would be to control the material synthesis for samples that are more compatible with the coating process for filters of more homogenous distribution of coated nanotubes, thus improved performance in capturing bacterial cells. On the other hand, the opportunities are such that there are still much room for improvements from the mostly proof-of-concept results in this study.

Figure 4: Representative SEM images of coated and un-coated filters, (A) a filter coated with cross linked PPEI-EI(5000)-MWNTs, (B) a filter coated with PPEI-EI(5000)-MWNTs, and (C) an un-coated filter. (A) A 3 μm pore sized filter and (B and C) 1.2 μm pore sized filters.

Similar SEM imaging conditions were applied to a direct examination on the coated filters with captured bacterial cells. Shown in Fig. 5 are representative SEM images of *E. coli* cells captured on PPEI-EI-MWNT-coated filters, which suggest that the functionalized MWNTs retained bacterial cells in several possible ways. For the first way, Fig. 5A indicates that *E. coli* cells could be trapped by their mostly sitting on top of the nanotubes, similar to what was observed in some previously reported studies on using CNTs coated filters for filtration of bacterial cells. The nanotubes on the filter apparently blocked the pores, so that the cells approaching the filter in a flow were trapped and retained on top of the coated filter. For the second way, Fig. 5B shows that *E. coli* cells were wrapped by PPEI-EI-MWNT species, and the wrapped cells were retained on the filters. Similar wrapping was observed when bacterial cells interacted with CNTs in solutions. The nanotubes coated on the filter were probably stirred up during the filtration process, so that the cells approaching the filter could be “mixed” with the nanotubes to result in the wrapping. The wrapped cells were thus aggregated and retained on the filter. The seemingly more complex way depicted in Fig. 5C might be considered as something deviated from the second way shown in Fig. 5B. This observation also brought up an attention that some of the PPEI-EI-MWNT samples may not adhere well to the filters by direct coating. As expected, the loss of polymer-coated MWNTs during the filtration was observed, and it mostly happened during the first 1 mL of filtration. It was estimated that on a 3.0 μm filter, at a filtration rate of 0.5 mL min⁻¹, the loss of non-cross-linked PPEI-EI-MWNTs and cross-linked PPEI-EI-MWNTs was about 0.7–10% and 5.9–14.9%, respectively, when a total of 0.345 to 0.575 mg (approximately 1.25 to 0.75 μg mm⁻²) MWNTs was coated on the filters. On the 1.2 μm pore sized filters, the average loss was 0.1–3% less than on the 3.0 μm pore sized filters. Again, these observations indicated that an improvement in the coating for stable adhesion and a more homogenous distribution of nanotubes on the filter should be pursued in further investigation.

The capturing efficiency of the coated filters was found to be dependent on the coating density of PPEI-EI-MWNT on the filters, as well as the flow rate of bacterial solution in the filtration process (Fig. 6). For example, when the coating density of PPEI-EI-MWNT on the filters increased from 0.095 to 0.25 and to 0.75 μg mm⁻², the efficiency in terms of log reduction improved from 0.73 log to 2.04 log and to 4.66 log, respectively, at the constant flow rate of 0.5 mL min⁻¹. With the same coated

Figure 5: SEM images of *E. coli* cells captured on filters coated with PPEI-EI-MWNTs. (A) and (B) PPEI-EI(5000)-MWNTs coated filters with 1.2 μm pores, and (C) cross-linked PPEI-EI-MWNTs coated filters with 3.0 μm pore.
filters, when the flow rate decreased to 0.25 mL min\(^{-1}\), the efficiency increased significantly to 1.61 to 4.32 and to >6 log, respectively. The results demonstrated that the coating of PPEI-EI-MWNT on filters could be tuned to alter the number of bacterial cells be captured, including also the possibility to select and optimize the coating parameters and filtration conditions to capture all bacterial cells from its original solution. However, the dependency of capture efficiency to coating density and flow rate for the cross-linked PPEI-EI-MWNT coated filters was not obvious. This again suggested that the coating inhomogeneity of the cross-linked samples on the filter rather than the density affected more significantly the capture efficiency. Certainly, besides the factors examined here, there are other factors that would affect the capture efficiency of the coated filter, which in general would include the factors related to the coating of PPEI-EI-MWNTs to the filters and the factors related to the interactions between the coated PPEI-EI-MWNTs and bacterial cells. The former may include, but not limited to the material nature of filters, the synthesis process of PPEI-EI-MWNTs, and the coating methods; whereas the latter may include the nature of PPEI-EI-MWNTs, bacterial species, bacterial shape, pH and salinity of the medium, and the presence/absence of other inorganic/organic compounds. Detailed investigations on the effect of these factors on the filtration efficiency would be necessary in further studies.

In the filtration through filters of pores larger than the bacterial cell dimension, the coating with functionalized MWNTs apparently served the desired function of capturing the bacterial cells. The functionalization and associated dispersibility of the nanotubes in solvent systems not only enabled the processing for the filter-coating but also facilitated the formation of nanotube networks on the filters, as designed to modify the large filter pores for cell retention. The results reported here have validated the approach, though the performance has not been improved substantially from those from the previously reported uses of un-modified MWNTs, SWNTs, or SWNTs-MWNTs hybrid coated filters.\(^{41,43,44}\) Nevertheless, the results have indeed revealed the potential of using filters coated with CNTs for capturing bacterial cells in simple filtration processing. For further performance improvements towards quantitatively and selectively capturing bacterial cells, the functionalized nanotube samples will have to be prepared such that they are sufficiently large in individual species yet still dispersible to be compatible with the coating processing. Again, the coating should be nanotube networks microscopically, for which the challenge would be an improved dispersion during and after the coating. The wetting characteristics of the filter surface might be an important issue, as it could have contributed to the observed inhomogeneous distribution of the nanotubes on the filter. In fact, it was noted in our tests that the hydrophobic filters (PTFE fluorophore filters from EMD Millipore) were not suitable for coating with the hydrophilic PPEI-EI-MWNTs. No reproducible results were achieved even with the aid of vacuum for the coating of PPEI-EI-MWNTs on PTFE filters. Other coating protocols will be explored in further investigations.

As reported previously, filters coated with mostly naked CNTs (without surface functionalization) served a noticeable function in inactivating pathogens. Here the polymer-functionalized CNTs and their coated filters may not have such a function, rather just retaining the status of the bacterial cells for their being separated from the samples/matrixes. Therefore, there may be opportunities for these filters as a good platform to be incorporated into subsequent detection methods designed for efficient screening of bacterial pathogens.

**Conclusions**

This study evaluated several PPEI-EI-MWNTs samples as coating materials on commercial hydrophilic polycarbonate filters for capturing of bacterial pathogens in aqueous solution. The results demonstrated that the coating of PPEI-EI-MWNTs on the filters effectively enhanced the efficiency of bacterial capture compared with un-coated filters. Bacterial capture efficiencies of >4 log, up to 6 log or larger in some experimental configurations, were achieved by the combination of coating density of PPEI-EI-MWNTs and the flow rate, which is comparable to or better than the performance of existing capture methods. The cross-linked PPEI-EI-MWNTs coated filters were unexpectedly less efficient than those with PPEI-EI-MWNT samples without the cross-linking, likely due to the poorer dispersibility of the cross-linked samples and thus their less homogeneous coating on filters. It was found that the bacterial capture efficiency of the PPEI-EI-MWNTs coated filters depended significantly on the properties of the PPEI-EI-MWNTs coating, as the dispersibility,
absorbitivity, and nanoscale details of the PPEI-EI-MWNTs were obvious factors that affected the distribution of nanotubes on the filter, the tightness of the coating nanotubes on the filter, and the interactions between the nanotubes and bacterial cells. This study has demonstrated that the coating of filters with functionalized carbon nanotubes represents a promising approach for effective capture of bacterial pathogens, and the capture step can potentially be incorporated into detection schemes to improve pathogen detection, especially for samples with low pathogen concentrations.

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Visible-Light Photoconversion of Carbon Dioxide into Organic Acids in an Aqueous Solution of Carbon Dots

Sushant Sahu,† Yamin Liu,† Ping Wang,† Christopher E. Bunker,*‡ K. A. Shiral Fernando,§ William K. Lewis,§ Elena A. Guliants,§ Fan Yang,† Jinping Wang,† and Ya-Ping Sun*†

†Department of Chemistry and Laboratory for Emerging Materials and Technology, Clemson University, Clemson, South Carolina 29634, United States
‡Air Force Research Laboratory, Propulsion Directorate, Wright-Patterson Air Force Base, Dayton, Ohio 45433, United States
§University of Dayton Research Institute, Sensors Technology Office, Dayton, Ohio 45469, United States

ABSTRACT: Carbon “quantum” dots (or carbon dots) have emerged as a new class of optical nanomaterials. Beyond the widely reported bright fluorescence emissions in carbon dots, their excellent photoinduced redox properties that resemble those found in conventional semiconductor nanostructures are equally valuable, with photon−electron conversion applications from photovoltaics to CO2 photocatalytic reduction. In this work we used gold-doped carbon dots from controlled synthesis as water-soluble catalysts for a closer examination of the visible-light photoconversion of CO2 into small organic acids, including acetic acid (for which the reduction requires many more electrons than that for formic acid) and, more interestingly, for the significantly enhanced photoconversion with higher CO2 pressures over an aqueous solution of the photocatalysts. The results demonstrate the nanoscale semiconductor-equivalent nature of carbon dots, with excellent potential in energy conversion applications.

INTRODUCTION

The level of carbon dioxide (CO2) in the atmosphere and its significant environmental implications have generated much concern, promoting the development of various carbon sequestration strategies. Photocatalytic conversion is obviously a compelling approach in this regard, in which CO2 is reduced back to hydrocarbon fuels. Even more desirable is the use of solar energy for the photoreduction, which presents a major challenge for the effective photon harvesting by the catalysts across the solar spectrum.1,2 Among widely employed photocatalysts for the purpose of CO2 reduction have been nanoscale wide-bandgap semiconductors such as titanium dioxide (TiO2) and cadmium sulfide (CdS) nanoparticles.1–4 However, as limited by their bandgap transitions, these nanomaterials are generally ineffective in harvesting visible photons over a broad spectral range. Thus, various enhancement approaches including the use of doped TiO2 nanotubes and/or dye sensitization to extend the absorption of the photocatalysts into the visible have been developed, with some significant successes. For example, Feng et al. prepared TiO2 nanotube arrays via the anodic oxidation of titanium foil in an electrolyte.5 The arrays under sunlight could catalyze the reduction of CO2 into methane, and the reduction efficiency could be enhanced substantially after the arrays were “coated” with ultrafine platinum nanoparticles.5 Woolerton et al. used enzyme-modified TiO2 nanoparticles (Degussa P25) with a ruthenium bipyridyl complex as a visible-light photosensitizer for the CO2 photocatalytic reduction.6 Asi et al. synthesized a nanocomposite of TiO2 with silver bromide for the visible-light (420 nm and longer) photoreduction of CO2.7 More recently, Cao et al. took a rather different approach to using surface-functionalized small carbon nanoparticles, dubbed “carbon dots” (Figure 1),8,9 for the absorption of visible photons to drive photocatalytic processes.10 In the photoreduction of CO2, the carbon dots were surface-doped with a...
small amount of gold or platinum metal, which was designed to concentrate the photogenerated electrons, similar to what was widely practiced in the development of conventional nanoscale semiconductor-based photocatalysts.4 The photocatalytic conversion of CO2 was probed by quantifying the formation of formic acid as a significant product10 from which the estimated quantum yields for the photoreduction were substantial in reference to those achieved in the literature with the use of semiconductor nanoparticles as photocatalysts.3,4,10 More importantly in terms of a primary purpose in that study to understand the photoexcited state processes in small carbon nanoparticles,10 the photocatalytic reduction results served to confirm the presence of photoinduced charge separation in carbon dots, as already suggested by the fluorescence quenching results of carbon dots with either electron donors or acceptors.9,11

Beyond mechanistic implications (with some other carbon-based photocatalytic systems12−14), the previous study opened the possibility for carbon dots to serve as a new platform of potent photocatalysts for more efficient CO2 photoreduction. However, because it is a new platform, there is naturally the demand for more experimental results for further validation. In the work reported here, we used gold-doped carbon dots from better-controlled synthesis as aqueous soluble catalysts for a closer examination of the visible-light photocconversion of CO2 into small organic acids, including acetic acid (whose formation requires many more electrons than that of formic acid).15 and more interestingly for the significantly enhanced photoconversion with higher CO2 pressures over an aqueous solution of the catalysts. The results from the pressure-dependent (and thus CO2 concentration-dependent) study under otherwise the same experimental conditions are particularly valuable in terms of confirming the participation of CO2 in the reaction as the source of the converted organic acids and also the photocatalytic functions of the carbon dots.

■ RESULTS AND DISCUSSION

The photocatalytic reactions were carried out in aqueous solutions under pressurized CO2 conditions. In the catalyst preparation, the fluorescent carbon dots with the core carbon nanoparticles functionalized by oligomeric poly(ethylene glycol) diamine (PEG1500N, Figure 1) were synthesized under more controlled conditions (primarily more precise temperature control in the functionalization reaction) for more effective carbon particle surface passivation and the associated bright fluorescence emissions, as reported previously.16 These carbon dots in aqueous solution exhibited fluorescence quantum yields of around 20% at 400−450 nm excitation, comparable to or better than what were observed in other batches of similarly synthesized PEGylated carbon dots in previous studies.16 For the photochemical doping of the carbon dots by gold metal, the aqueous solution of the carbon dots was irradiated in the presence of gold salt HAuCl4, where the electrons from the photoinduced charge separation in the carbon dots were likely responsible for the reductive formation of gold metal (Figure 2).17 Since the radiative recombination of the surface-confined electrons and holes is believed to be responsible for the observed fluorescence emission in carbon dots,9 the metal doping was accompanied by rapidly diminishing fluorescence intensities in the solution (Figure 2), as expected.17

The doping level of gold on carbon dots was monitored by the emergence of the gold plasmon band in optical absorption spectra (Figure 3). The association of the nanoscale gold and carbon dots, namely, the doping, could be confirmed directly by the transmission electron microscopy (TEM) imaging comparatively in both transmission and Z-contrast modes (Figure 3) and also indirectly by the observed complete fluorescence quenching due to the doped gold interfering with the emissive processes in carbon dots (Figure 2).17 The gold-doped carbon dots used as photocatalysts in this study generally had a gold-to-carbon (in the nanoparticle core) molar ratio of around 1:100.

The photocatalytic reduction of CO2 in an aqueous solution of the gold-doped carbon dots was carried out at ambient temperature (25 °C) in high-pressure cylindrical optical cells (Figure 2) capable of taking the CO2 pressure up to at least 2000 psia, corresponding to an aqueous CO2 concentration of up to 1.37 M (calculated according to data available in the literature).19 Small organic acids in the reaction mixture were targeted for detection and analysis quantitatively. The acids were harvested by distilling the reaction mixture into a basic aqueous solution (pH ~11), followed by recovering the salts thus formed for NMR characterization and other analyses.

The 1H and 13C NMR results suggested a substantial presence of formic acid as a significant product of the CO2 photoreduction, as generally known in the literature,3,20−24 and also acetic acid.15,25−30 Separately, the solution used in the NMR measurements was acidified (pH ~3) to convert the salts to corresponding acids for GC-MS analyses, from which the results also identified formic acid and acetic acid, as expected. In the literature,15,25−30 acetic acid has been identified as a product in CO2 photoreduction with the use of other photocatalysts, though quantity-wise from minor to negligible in almost all studies. Therefore, since the photocatalytic activities of carbon dots are due to the photochemical processes...
in the core carbon nanoparticles, the concern was the potential involvement of the carbon in the core as a source of carbon atoms in the observed acetic acid. In control experiments, $^{13}$C-labeled CO$_2$ (NaH$^{13}$CO$_3$ with 99% $^{13}$C as a source) was used in the same photoreduction reactions. The $^{13}$C NMR results for the formic acid and acetic acid suggested major enhancement effects in both, with the corresponding $^{13}$C NMR signals readily detected in many fewer scans than what was required for samples without the $^{13}$C enrichment, due to the products from $^{13}$C-labeled CO$_2$ (or, more specifically, H$^{13}$COOH and $^{13}$CH$_3^{13}$COOH).

Other experimental evidence against any potential involvement of the carbon atoms in carbon dots included the observation that the use of the same carbon dots without a gold coating for the photocatalytic conversion of CO$_2$ resulted in much lower yields for both formic acid and acetic acid. Mechanistically, it is believed that the photoexcitation of carbon dots results in efficient charge separation, with the generated electrons and holes trapped at surface sites on the carbon dots. The electrons and holes would otherwise recombine radiatively for the fluorescence emission in carbon dots, but the metal doping interrupted the radiative recombination by scavenging the electrons (Figure 2).9,17 Here the doped gold could apparently harvest and concentrate the photogenerated electrons in the carbon dots, thus more efficiently in the photocatalytic activities for the CO$_2$ reduction, as generally understood in the literature.2,4

In a further examination of the involvement of water as a source of hydrogen in the formed organic acids, the photocatalytic reduction of CO$_2$ was performed in a deuterated water solution under the same experimental conditions, followed by the same product collection and isolation procedures. In the subsequent $^1H$ NMR characterization (regular water as the solvent), the expected DCOO$^-$ and CD$_3$COO$^-$ NMR signals could readily be detected at 8.47 and 1.89 ppm, respectively, suggesting the participation of D$_2$O in the photoreduction. A mechanism known in the literature in which the involvement of water in the photocatalytic conversion of CO$_2$ might be associated with a two-step reduction process, first the photocatalytic splitting of water for atomic hydrogen and then the addition of hydrogen to CO$_2$.3,31

The observed deuteration of both formic acid and acetic acid from the reaction in D$_2$O is consistent with such a mechanism and also reaffirms the mechanistic connection between the formation of the two organic acids in the photocatalytic reduction of CO$_2$.

There are several ways to evaluate the performance of catalysts in the CO$_2$ photoreduction reaction,4 among which a more popular one has been the $R$ value measuring the amount of products produced per hour of light illumination for a specific amount of photocatalyst

\[
R = \frac{W_P}{W_C}\quad (1)
\]

where $W_P$ is the amount of photoproducts in millimoles (mmol), $t$ is the photoirradiation time in hours (h), and $W_C$ is the amount of photocatalyst used in grams (g). For the gold-doped carbon dots as photocatalysts in this work, quantitatively the amounts of the small organic acids produced in the photoreduction were subject to some variations with experimental conditions such as different batches or quantities of catalysts and reactor setups, which also affected the relative populations of the two acids in the reaction mixtures. Nevertheless, with the short-path optical cell as a high-pressure photoreactor under a specifically controlled condition (CO$_2$ pressure of 700 psia and photoirradiation in the 405−720 nm wavelength range for 4 h), the observed $R$ values for formic acid and acetic acid were 1.2 and 0.06 mmol h$^{-1}$ g$^{-1}$, respectively. The performance of the former compares favorably to what has been reported in the literature for other photocatalysts. For example, in the study by Zhao et al. on CO$_2$ photoreduction with the cobalt phthalocyanine−TiO$_2$ nanocomposite as the photocatalyst and visible-light irradiation, the best $R$ value for formic acid was about 0.15 mmol h$^{-1}$ g$^{-1}$; John and Kisch used ZnS nanoparticles loaded on a silica matrix as photocatalysts for CO$_2$ reduction with 2,5-dihydroxyfuran as a reducing agent and UV-light irradiation, achieving a formate production rate of about 0.29 mmol/h (or approximately 0.6 mmol h$^{-1}$ g$^{-1}$ in terms of the $R$ value).21

The significant acetic acid production with the use of gold-doped carbon dots as photocatalysts is somewhat unique, as this is generally a very minor product in the CO$_2$ photoreduction with other catalysts.15,25−30 The previously highest $R$ value for acetic acid in the literature was from the work of Pathak et al., where well-dispersed TiO$_2$ nanoparticles were used as photocatalysts for UV-light reduction in supercritical CO$_2$ under high pressure, though the estimated $R$ value was still rather small (on the order of 0.006 mmol h$^{-1}$ g$^{-1}$).28 Irvine et al. also observed a significant amount of acetic acid in their colloidal CdS-based photocatalytic CO$_2$ reduction in aqueous NaHCO$_3$ with sulfitre or hydroquinone as a hole acceptor (320−580 nm photoirradiation), with an estimated acetic acid production rate of about 0.00125 mM/h (or an $R$ value of...
about 0.004 mmol h\(^{-1}\) g\(^{-1}\)).\(^{26}\) The relatively more efficient formation of acetic acid in the work reported here reflects upon the effectiveness of the gold-doped carbon dots as photocatalysts for visible-light-driven CO\(_2\) conversion, as the reduction to acetic acid requires overall eight electrons, regardless of any detailed mechanisms.\(^{35}\)

\[
2\text{CO}_2 + 8\text{H}^+ + 8e^- \rightarrow \text{CH}_2\text{COOH} + 2\text{H}_2\text{O} \quad (2)
\]

Again in studies already reported in the literature,\(^{15,25-30}\) acetic acid has generally been a very minor or negligible product in CO\(_2\) photoconversion. Therefore, the formation mechanism for the product is poorly understood, except for a general acknowledgment of the mechanistic complexity. Among the three proposed mechanisms in the literature,\(^{4,26,29,32}\) two assumed the dimerization of initially reduction-generated radical or radical anion species as a key step in the formation of acetic acid, specifically, OHC\(^-\) or CO\(_2\)\(^-\), dimerized to OHC\(=\)CHO or \(=\text{OOC}=\text{COO}\), respectively, followed by further photoreduction.\(^{26,32}\) The other mechanism in the acetic acid formation called for the reductive coupling of methanol and COOH, both of which are from initial steps in the CO\(_2\) photoreduction process.\(^{29}\) A common theme is that acetic acid shares intermediates with formic acid and/or reacts further from the latter, signifying the important role of formic acid in the understanding of the photocatalytic CO\(_2\) conversion.

For a more systematic evaluation of the photoconversion under different CO\(_2\) pressures, the reactions were carried out in the long-path high-pressure optical cell (thus a larger reactor volume for more samples/product quantities and thus relatively improved accuracy) under otherwise the same experimental conditions for all CO\(_2\) pressures. The formic acid production obviously increased with the increasing CO\(_2\) pressure (Figure 4), mainly because the CO\(_2\) concentration in aqueous solution increases with increasing pressure. At 1900 psia, the amount of formic acid was about an order of magnitude higher than that produced under ambient CO\(_2\) pressure, which should correspond roughly to the same magnitude of increase in the photocatalytic reaction quantum yield for the formic acid formation (around 0.3% in the CO\(_2\)-saturated aqueous solution under ambient pressure,\(^{10}\) estimated by using the method of Mallouk and coworkers\(^{33,34}\)). The strong CO\(_2\) pressure dependence might be understood in terms of the importance of the initial interactions between the photoexcited catalysts and CO\(_2\) molecules in the conversion to formic acid, with more CO\(_2\) molecules in the aqueous solution under a higher CO\(_2\) pressure in the optical cell and/or the involvement of more complex multistep processes in the photoconversion reaction. Shown in Figure 5 is a comparison of the \(R\) values for formic acid and acetic acid from the experiments at different CO\(_2\) pressures.

![Figure 4](image1.png)

**Figure 4.** Results in terms of formic acid concentrations in the reaction mixtures from a series of photoconversion reactions in aqueous solutions of the carbon dots under different CO\(_2\) pressures (in the long-path optical cell as the high-pressure reactor).

The gold-doped carbon dots as photocatalysts were apparently stable in terms of their optical properties and catalytic activities. For example, upon continued photo-irradiation for 6 h or longer in the photocatalytic reaction for CO\(_2\) conversion, the optical absorption spectrum of the photocatalysts postreaction exhibited no significant changes from that prereaction (Figure 3). The photocatalysts could also be recovered from the reaction mixtures and reused in the subsequent photoreduction reactions of CO\(_2\). The difference between the use of new and recovered photocatalysts is generally within the typical experimental variation (the changes in the results with the use of new catalysts in several experiments under the same conditions).

In summary, carbon dots with a gold coating and thus diminished fluorescence emission are potent photocatalysts for the conversion of CO\(_2\) to small organic acids. The formation of a significant amount of acetic acid, which requires many electrons in the photoreduction, reflects upon the effectiveness of the carbon dots as photocatalysts. The photocatalytic functions are apparently not limited to the carbon dots synthesized in this study, as even those prepared with carbon soot from overcooked barbequed meat exhibited similar activities.\(^{35}\) The substantially enhanced photoconversion in aqueous solution of the catalysts under higher CO\(_2\) pressures is not only important mechanistically, suggesting the role of the CO\(_2\) concentration in the harvesting of photogenerated electrons and thus the photoconversion quantum yield, but also valuable technologically, with pressurized CO\(_2\) as a more
favorable reaction condition for larger quantities of the photoproducts.

**Experimental Section**

**Materials.** Carbon nanopowder (purity >99%) was purchased from Sigma-Aldrich, bis(3-aminopropyl)-terminated oligomeric poly(ethylene glycol) of average molecular weight ∼1500 (PEG1500) was purchased from Anvia Chemicals, CO₂ gas (purity >99.5%) was purchased from Airgas, and hydrogen tetrachloroaurate (HAuCl₄) was purchased from Anvia Chemicals. Imidazole and fumaric acid were obtained from Aldrich Chemicals, thionyl chloride (purity >99%) was obtained from Alfa Aesar, nitric acid was obtained from VWR, and 13C-enriched sodium bicarbonate (NaH¹³CO₃, purity 97% and ¹³C content 99%) and D₂O purchased from Sigma-Aldrich, and hydrogen tetrachloroaurate (HAuCl₄) was purchased from Anvia Chemicals, thionyl chloride (purity >99%) was obtained from Alfa Aesar, nitric acid was obtained from VWR, and ¹³C-enriched sodium bicarbonate (NaH¹³CO₃, purity 97% and ¹³C content 99%) and D₂O were obtained from Cambridge Isotope Laboratories. HPLC-grade solvents isopropanol, methanol, and phosphoric acid were supplied by Fisher Scientific, and dialysis membrane tubing (MWCO ~500) was supplied by Spectrum Laboratories. Water was deionized and purified by being passed through a Labconco WaterPros water purification system.

**Measurements.** A Baxter Megafuge (model 2630), Eppendorf (model 5417 R), and Beckman-Coulter ultracentrifuge (Optima L90K with a type 90 Ti fixed-angle rotor) were used for centrifugation at various g values. Optical absorption spectra were recorded on a Shimadzu UV-2501PC spectrophotometer. NMR measurements were carried out on Bruker (500 and 300 MHz) and JEOL (500 MHz) NMR spectrometers. GC-MS analyses were performed on a Shimadzu GC-MS instrument (model QP 2010) with an Rxi-XLB/or Rtx-5MS column and an electron ionization (EI) MS detector. Transmission electron microscopy (TEM) images were obtained on Hitachi 9500 TEM and HD-2000 scanning-TEM systems.

**Photocatalysts.** In the synthesis of PEG₁₅₀₀₀-functionalized carbon dots, an as-supplied carbon nanopowder sample (1 g) was refluxed in an aqueous nitric acid solution (5 M, 90 mL) for 48 h. The reaction mixture back at room temperature was dialyzed against fresh water and then centrifuged at 30000 g to retain the supernatant. The recovered sample containing primarily small carbon nanoparticles was refluxed in neat thionyl chloride for 12 h, followed by the removal of excess thionyl chloride under nitrogen. The post-treatment carbon particle sample (100 mg) was mixed well with carefully dried PEG₁₅₀₀₀ (1 g) in a flask, heated to 140 °C, and stirred at constant temperature under nitrogen for 72 h. The reaction mixture was cooled to room temperature, dispersed in water, and then centrifuged at 20 000 g to retain the dark supernatant as an aqueous solution of the as-prepared carbon dots.

For the metal doping of carbon dots, an aqueous dispersion of the carbon dots was mixed with an aqueous solution of gold compound (HAuCl₄), and the mixture was irradiated with visible light. The reaction mixture was cooled to room temperature, dispersed in water, and then centrifuged at 20 000 g to retain the supernatant. The as-prepared sample containing primarily small carbon nanoparticles was recovered sample containing primarily small carbon nanoparticles was refluxed in neat thionyl chloride for 12 h, followed by the removal of excess thionyl chloride under nitrogen. The post-treatment carbon particle sample (100 mg) was mixed well with carefully dried PEG₁₅₀₀₀ (1 g) in a flask, heated to 140 °C, and stirred at constant temperature under nitrogen for 72 h. The reaction mixture was cooled to room temperature, dispersed in water, and then centrifuged at 20 000 g to retain the dark supernatant as an aqueous solution of the as-prepared carbon dots.

**Photocatalytic Reactions.** The CO₂ photocatalytic reduction experiments under different CO₂ pressures were performed on a setup consisting of a 1 kW xenon arc source coupled with a “hot filter” to eliminate both infrared and UV radiation (transparent only in the 405–720 nm spectral range). Two stainless steel cylindrical optical cells with flat front and back sapphire windows (2.5 cm in diameter, sealed with teflon O-rings) were used as photochemical reactors with a pressure limit of at least 2000 psia. The short-path cell had an optical path length of 16 mm (∼4 mL in reactor volume), and the long-path cell, 80 mm (∼20 mL in reactor volume). In a typical experiment, the cell was first loaded (not completely full) with an aqueous dispersion of the photocatalysts, purged with high-purity nitrogen gas under ambient condition, and then sealed. Pressurized CO₂ (in a syringe pump) was introduced into the sealed cell through the metal tubing and valve until the desired pressure in the cell was achieved, and the actual pressure upon stabilization of the system was measured and recorded by using a precision pressure gauge (Heise).

Some CO₂ photocatalytic reduction experiments under ambient pressure were carried out by using an ACE Glass immersion-well photochemistry apparatus equipped with a 450 W medium-pressure Hanovia lamp coupled with a cycling water filter and a glass or solution filter. An aqueous dispersion of the photocatalysts in the photochemistry apparatus was purged first with high-purity nitrogen gas and then with CO₂ gas (for about 60 min) for saturation, followed by photolysis.

Aqueous NaHCO₃ or NaH¹³CO₃ solution (up to 80 mM, mimicking mildly acidic conditions of pH ∼4.5–5.5) was used as CO₂ or ¹³C-enriched CO₂ in the same photocatalytic reactions.

Isopropanol was added in some of the experiments described above as a sacrificial electron donor, though no meaningful difference was found in the outcome of the photocatalytic reduction (probably due to the fact that PEG molecules of similar structural elements to those in isopropanol were already in the reaction system).

**Photoproduc t Characterization and Analysis.** The aqueous reaction mixture post-photolysis was collected via slow depressurization from the high-pressure optical cell into a cooled flask, followed by short-path distillation into an aqueous NaOH solution (pH ∼11) for the organic acids in the reaction mixture to be trapped as salts. Upon the removal of water via evaporation, the resulting solid sample was characterized by using NMR and GC-MS methods. Formic acid and acetic acid (or formate and acetate in some measurements) were identified, confirmed, and quantified. Imidazole and fumaric acid were used as internal standards in the ¹H NMR quantification measurements.

**Author Information**

**Corresponding Authors**

*E-mail: christopher.bunker@wpafb.af.mil.
E-mail: syaping@clemson.edu.

**Notes**

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**References**


Toward Structurally Defined Carbon Dots as Ultracompact Fluorescent Probes

Gregory Ethan LeCroy,† Sumit Kumar Sonkar,† Fan Yang,† L. Monica Veca,§,* Ping Wang,† Kenneth N. Tackett, II,† Jing-Jiang Yu,§ Eugeniu Vasile,‡ Haijun Qian,‡ Yamin Liu,† Pengju (George) Luo,† and Ya-Ping Sun†,*

†Department of Chemistry and Laboratory for Emerging Materials and Technology, Clemson University, Clemson, South Carolina 29634, United States, ‡National Institute for Research and Development in Microtechnologies, IMT-Bucharest, Bucharest 077190, Romania, §Nanotechnology Measurements Division, Agilent Technologies, Inc., Chandler, Arizona 85226, United States, and †Department of Oxide Materials and Nanomaterials, Faculty of Applied Chemistry and Material Science, University Politehnica of Bucharest, Bucharest, Romania

ABSTRACT There has been much discussion on the need to develop fluorescent quantum dots (QDs) as ultracompact probes, with overall size profiles comparable to those of the genetically encoded fluorescent tags. In the use of conventional semiconductor QDs for such a purpose, the beautifully displayed dependence of fluorescence color on the particle diameter becomes a limitation. More recently, carbon dots have emerged as a new platform of QD-like fluorescent nanomaterials. The optical absorption and fluorescence emissions in carbon dots are not bandgap in origin, different from those in conventional semiconductor QDs. The absence of any theoretically defined fluorescence color - dot size relationships in carbon dots may actually be exploited as a unique advantage in the size reduction toward having carbon dots serve as ultracompact QD-like fluorescence probes. Here we report on carbon dots of less than 5 nm in the overall dot diameter with the use of 2,2’-(ethylenedioxy)bis(ethylamine) (EDA) molecules for the carbon particle surface passivation. The EDA-carbon dots were found to be brightly fluorescent, especially over the spectral range of green fluorescent protein. These aqueous soluble smaller carbon dots also enabled more quantitative characterizations, including the use of solution-phase NMR techniques, and the results suggested that the dot structures were relatively simple and better-defined. The potential for these smaller carbon dots to serve as fluorescence probes of overall sizes comparable to those of fluorescent proteins is discussed.

KEYWORDS: carbon dots · fluorescence · green fluorescent protein (GFP) · quantum dots · bioimaging · molecular probes · ultracompact probes · functionalized nanoparticles

Fluorescent semiconductor nanocrystals, generally referred to as quantum dots (QDs) for the quantum confinement effect in these nanomaterials, have attracted much attention for their serving as optical probes in biomedical and other applications.1,2 Among the most popular QDs, some of which are now commercially available, are those based on cadmium salts, especially CdSe/ZnS core–shell nanostructures with various surface coatings for organic or aqueous compatibilities.3–5 Strong cases have been made in the literature on using QDs to replace organic dyes and in some applications genetically encoded fluorescent tags.6,7 Among many widely considered advantageous properties of conventional semiconductor QDs, a unique feature due to the quantum confinement effect is the beautiful display of different fluorescence colors for QDs of different sizes. However, the defined color–size dependence also limits any dot size variation for a specific fluorescence color. For the more established CdSe/ZnS QDs with the necessary surface coating (for solubility and/or compatibility needs) as fluorescence probes, the probe size profiles are typically on the order of 10 nm in diameter.6–8 Therefore, there has been increasing interest in a reduction of the probe size, targeting similar size profiles to those of many commonly used fluorescent proteins (green fluorescent protein, or GFP, for example),9 for which strategies such as the use of a thinner coating on the QD surface have been pursued.1,10–12

In terms of application potential, ultracompact probes (referring to structurally compact and very small in the overall probe size) offer additional and sometimes unique
opportunities, with specific biologically relevant examples including their less interference with or perturbation to the biological events in the cells being probed\textsuperscript{13–15} and more favorable renal clearance, for which a threshold probe size around 5.5 nm was determined.\textsuperscript{16} There were also studies in which issues with the use of larger nanoparticles were identified, such as their accumulation in leaky vasculature or in solid tumors, thus potentially inhibiting clearance and increasing the likelihood of long-term toxicity effect.\textsuperscript{17–19}

Most of the recently pursued ultracompact probes were based on metal or metal oxide nanoparticles, especially sub-5 nm gold nanoparticles\textsuperscript{20,21} and gadolinium or iron oxide nanoparticles for MRI tracking applications.\textsuperscript{22,23} As a demonstration on the critical effect of probe size, gold nanoparticles of 2.4 nm in diameter were found in the cell nucleus, 5.5–8.2 nm in the cytoplasm, and 16 nm or larger mostly outside the cell.\textsuperscript{21}

Carbon dots have emerged as a new platform for QD-like fluorescent nanomaterials,\textsuperscript{24} with competitive optical performance under one- and multiphoton excitation conditions and their being generally nontoxic in nature.\textsuperscript{25–39} Presently more fluorescent carbon dots are those of small carbon nanoparticles with the particle surface passivated by chemical functionalization with oligomeric or polymeric species. For example, with the oligomeric poly(ethylene glycol) diamine (PEG\textsubscript{1500N}) as surface passivation agent, the PEG\textsubscript{1500N}–carbon dots exhibited fluorescence quantum yields of more than 50\%.\textsuperscript{34} Mechanistically, both the optical absorption and fluorescence emissions in carbon dots are not band gap in origin, due instead to $\pi$-plasmon and radiative recombination of the surface-confined electrons and holes, respectively,\textsuperscript{39,40} different from those in conventional semiconductor QDs. The absence of any theoretically defined fluorescence color–dot size relationships in carbon dots may actually be exploited as a unique advantage in the size reduction toward having carbon dots serve as ultracompact QD-like fluorescence probes. More specifically, there has been extensive recent discussion on the push to reduce the sizes of fluorescence probes (mostly those based on conventional semiconductor QDs with the necessary surface coating) to less than 5 nm in diameter.\textsuperscript{9} In this regard, the PEG\textsubscript{1500N}–carbon dots referred to above had an average core carbon nanoparticle size of about 3 nm,\textsuperscript{34} but the overall dot profiles were on average significantly larger due to the use of the relatively large PEG\textsubscript{1500N} molecules (average molecular weight $\sim$1500) for carbon particle surface passivation via chemical functionalization. The expectation was such that a reduction in the size of the surface functionalization molecules would not only add less to the overall profile (the core carbon nanoparticle + surface passivation layer) of each carbon dot but also be more selective toward the solubilization of smaller carbon nanoparticles in the dot synthesis, resulting in smaller carbon dots and their corresponding fluorescence probes.

Here we report on carbon dots of less than 5 nm in overall dot diameter with the use of 2,2’-(ethylenedioxy)bis(ethylamine) (EDA, H$_2$NCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$NH$_2$) molecules for carbon particle surface functionalization–passivation (Figure 1). The EDA–carbon dots were found to be brightly fluorescent, especially over the GFP spectral range. These aqueous soluble smaller carbon dots also enabled more quantitative characterizations, including the use of solution-phase NMR techniques, and the results suggested that the dot structures were relatively simple and better defined. The potential for these smaller carbon dots to serve as fluorescence probes of overall sizes comparable to those of fluorescent proteins (Figure 1)\textsuperscript{41–43} is also discussed.

RESULTS AND DISCUSSION

Precursor carbon nanoparticles were obtained from the commercially supplied sample of carbon nanoparticles in a procedure that included refluxing the as-supplied sample in an aqueous nitric acid solution, dialysis, centrifuging to retain the supernatant (with a higher population of smaller particles), and then the removal of water.\textsuperscript{34} The nanoparticles were functionalized with EDA molecules under amidation reaction conditions. Briefly, the sample of carbon nanoparticles was first refluxed in neat thionyl chloride and then mixed well with carefully dried EDA (liquid at ambient temperature). The mixture was heated to 120 °C and vigorously stirred for the reaction under nitrogen protection. The reaction mixture was dispersed in water for centrifugation to retain the supernatant, followed by purification through a gel column (packed in-house by using commercially supplied Sephadex G-100 gel)\textsuperscript{44} to isolate the targeted carbon dots (EDA-functionalized carbon nanoparticles, Figure 1) of bright fluorescence emissions. The carbon dots sample was further purified in dialysis against fresh water to remove residual small molecular species including free EDA molecules, yielding a clean aqueous solution of the purified carbon dots (designated as

![Figure 1. Cartoon illustration (left) of an EDA-carbon dot, which is essentially a special “core–shell” nanostructure with a small carbon nanoparticle as the core and a soft shell of tethered EDA molecules, and (right) green fluorescent protein with the size profile highlighted.](image-url)
EDA-carbon dots). According to results from the quantitative optical absorption measurement of the EDA-carbon dots (Figure 2), in which the amount of core carbon nanoparticles was calculated with the separately determined molar absorptivity values\textsuperscript{45} and the amount was compared to that of the precursor carbon nanoparticles, the estimated overall reaction yield was around 10\% (the percentage of the precursor carbon nanoparticles converted to the core carbon nanoparticles in the final purified EDA-carbon dots).

The EDA-carbon dots were characterized by using atomic force microscopy (AFM) for the determination of overall dot sizes. The AFM specimen was prepared by dropping an aqueous solution of a selected dot concentration onto the mica surface, followed by the evaporation of water. Shown in Figure 3 are representative AFM imaging results for the EDA-carbon dots, in which according to height analyses an overwhelming majority of the dots were less than 5 nm in diameter. Multiple AFM images were used in the same height analyses to produce a data set for about 280 dots, and as also shown in Figure 3, the statistical analysis with a simple Gaussian function yielded an average dot size of 4.1 nm. The results are consistent with the expectation that the EDA-carbon dots should be smaller than the PEG\textsubscript{1500N}-carbon dots, which averaged around 5 nm in diameter according to previous investigations\textsuperscript{34,39} The conclusion is also supported by the results from transmission electron microscopy (TEM) imaging experiments.

For the PEG\textsubscript{1500N}-carbon dots reported previously, the TEM imaging enabled the size characterization of the core carbon nanoparticles, averaging 3 nm in diameter,\textsuperscript{34} as the organic surface functionalization molecules (PEG\textsubscript{1500N}) were largely transparent in contrast with the carbon core. For some dots in which the core carbon nanoparticles were more crystalline, the size measurements could also benefit from the more defined lattice fringes (Figure 3). However, in order to apply the TEM imaging to the determination of overall size profiles that include the surface passivation layer in carbon dots (Figure 1), the approach of doping the dots with materials of high electron density has found some success.\textsuperscript{46} In this work the EDA-carbon dots were doped with gold metal in a simple photolysis procedure, with visible-light photoirradiation of the dots in an aqueous solution of the Au(III) compound HAuCl\textsubscript{4}.\textsuperscript{46} The gold-doped dots were readily detected in TEM imaging for the improved dispersion into individual dots on the TEM grid, in addition to the increased electron densities. The TEM images (Figure 3) thus obtained were consistent with the AFM results (Figure 3), which suggested that the EDA-carbon dots should be mostly less than 5 nm in diameter for the overall dot profiles including the surface passivation layer of tethered EDA molecules.

The optical transitions in carbon dots are due to the \(\pi\)-plasmon absorption of the core carbon nanoparticles, namely, the “chromophores” in the dots.\textsuperscript{45} The absorption (Figure 2) is relatively strong, with
the observed per-carbon molar absorptivities of $50 - 100 \text{M}_c \text{ atom}^{-1} \text{ cm}^{-1}$ in the 400–450 nm region, where $\text{M}_c \text{ atom}^{-1}$ denotes molar concentration in terms of carbon atoms in the core carbon nanoparticles (assuming no other elements) for the carbon dots in a solution. For a carbon core size of 3 nm in diameter, the number of carbon atoms in the core was estimated to be around 1700, thus per-dot molar absorptivities of approximately $85\,000 - 170\,000 \text{M}_c \text{ particle}^{-1} \text{ cm}^{-1}$ for the same wavelength region, where $\text{M}_c \text{ particle}^{-1}$ refers to the molar concentration of the carbon dots with 3 nm diameter carbon cores. The per-dot molar absorptivities should obviously be sensitive to the dot diameter. For example, the EDA-carbon dots with a carbon core of 3.5 nm in diameter would have absorptivities up to approximately $250\,000 \text{M}_c \text{ particle}^{-1} \text{ cm}^{-1}$.

These estimates accounted for only carbons in the nanoparticle cores (again the visible chromophores) in the carbon dots, with the carbons in EDA molecules excluded for their being nonabsorptive in the visible spectral region.

The fluorescence spectrum of the EDA-carbon dots in aqueous solution, with excitation at 400 nm, is also shown in Figure 2. It is relatively broad, similar to those of carbon dots with other surface-functionalization molecules. The green fluorescence emissions are associated with quantum yields around 30%, determined in reference to 9,10-bis(phenylethynyl)anthracene as a standard (quantum yield of unity, calibrated against the quinine sulfate standard). The fluorescence properties of the EDA-carbon dots were apparently stable with respect to further sample purification effort on removing any loosely attached EDA molecules from the carbon dots in vigorous dialysis. Both the fluorescence spectrum and quantum yield remained the same after the repeated dialysis procedures, suggesting that the aqueous dispersed EDA-carbon dots were structurally robust, with the EDA functionalization on the dot surface being either covalent or associated with bonding-like strong interactions. More generally, the EDA-carbon dots are similar to the more extensively studied PEG_1500N-carbon dots in terms of excellent chemical and photochemical stabilities.

In molecular imaging and related uses, the performance of fluorescence probes is often measured in terms of the relative brightness, expressed as (molar absorptivity) × (fluorescence quantum yield). For the EDA-carbon dots of the carbon core at 3 nm in diameter, the corresponding fluorescence probes of less than 5 nm in diameter and with green fluorescence emissions could have a relative brightness as high as 50 000. As a rough comparison, the relative brightness values quoted in the literature on the commonly used GFP and derivatives are on the order of 30 000.

Structurally an EDA-carbon dot is simply a small carbon nanoparticle with a thin layer of tethered EDA molecules on the particle surface (Figure 1). The aqueous dispersion of EDA-carbon dots is solution-equivalent in appearance and in properties, suitable for solution-phase NMR characterizations. The $^1\text{H}$ NMR spectrum of the EDA-carbon dots (Figure 4), due to the tethered EDA molecules on the dot surface, shows broader signals than those in the spectrum of free EDA, consistent with the reduced mobility of the EDA molecules attached to carbon nanoparticles. Also consistent with the attachment is the lower symmetry for the particle-bound EDA species, with $^1\text{H}$ NMR signals for the three sets of protons in free EDA (Figure 4, γ: singlet, β: triplet, and α: triplet) split into multiple peaks in two groups, one for γ and β protons and the other for α protons (Figure 4). The relative integrations between γ + β and α proton signals are about 2.3 to 1, larger than the theoretical ratio of 2 to 1. A question was then on the assignment of the signal around 3.5 ppm, namely, the possibility for its being due to α protons. Such a possibility could be eliminated on the basis of correlation spectroscopy (COSY) results, which clearly identified the coupling of the broad 3.5 ppm signal with those of the α protons. Therefore, an alternative explanation is such that the NMR signal integration for the α protons is distorted by these protons being closer to the core carbon nanoparticles in the carbon dots. The $^{13}\text{C}$ NMR spectrum of the EDA-carbon dots (Figure 4) shows similarly two groups of peaks, again one for γ and β carbons and the other for α carbons, but no meaningful peaks that could be assigned to the core carbon nanoparticles. Results from the FT-IR characterization were generally consistent with the NMR results. The carbon nanoparticles...
before the EDA functionalization exhibited only weak absorptions in the 1550–1750 cm⁻¹ region, suggesting the presence but low population of oxygen-containing moieties (such as carboxylic acids). These absorption features could still be identified in the spectrum of the EDA-carbon dots, although only to a rather limited extent due to their overlapping with the more substantial absorption of EDA over the same spectral region (1400–1700 cm⁻¹). Overall the FT-IR spectrum of the EDA-carbon dots is similar to that of EDA molecules in terms of major spectral features, but with some peak broadening probably due to the association of EDA with carbon nanoparticles in the carbon dots. Therefore, the FT-IR results were not as useful as desired in the elucidation of structural details, but overall did seem to suggest that there were no significant changes to the EDA chemical structure, such as those that might create visible chromophores, as a result of the functionalization chemistry (probably as expected for the rather mild reaction conditions). Further ¹³C NMR characterization was on the similarly prepared EDA-carbon dots with the core carbon nanoparticles ¹³C-enriched. Experimentally, the carbon soot sample containing ¹³C-enriched carbon nanoparticles was produced in the arc-discharge of two graphite rods, one of which was made hollow and then filled with a mixture of commercially supplied ¹³C powders and graphite cement. According to quantitative Raman spectral shift measurements, the precursor carbon nanoparticles for the functionalization with EDA molecules had a ¹³C content of 10–15%, and the same ¹³C enrichment in the resulting carbon dots should be expected. As compared in Figure 2, the absorption and fluorescence spectra of the ¹³C-enriched EDA-carbon dots are rather similar to those without the ¹³C enrichment. For the ¹³C NMR results of the ¹³C-enriched EDA-carbon dots, there are no additional peaks in the aliphatic region nor any detectable aromatic signals (Figure 4). The relatively weak but meaningful peaks in the 175–160 ppm region (Figure 4) may be assigned to carbonyl carbons on the core carbon nanoparticles. However, since smaller carbon nanoparticles are expected to have more diverse surface defects or carbon sites, the ¹³C NMR signals of the EDA-carbon dots, whose carbon cores are definitely at the smaller side, are subject to more significant broadening effects. Therefore, the observed carbonyl signals likely represent only some in the minority that could be detected. The results are informative in the sense that they are consistent with the expected diverse carbon environment on the core carbon nanoparticle surface.

It is interesting that the functionalization results for the precursor carbon nanoparticles without and with the ¹³C-enrichment were rather similar, as the nanoparticles were from different production methods, laser processing for the commercially supplied sample vs arc-discharge for the ¹³C-enriched sample. The former is generally somewhat more crystalline than the latter. Therefore, the nearly identical optical properties of their resulting EDA-carbon dots suggest stability with the synthesis method for more consistent production of the carbon dots from different sources of precursor carbon nanoparticles.

The EDA-carbon dots as ultracompact fluorescence probes, with overall size profiles of less than 5 nm in diameter, are not limited to green fluorescence only, with emissions also observed in other colors at different excitation wavelengths (Figure 5). Again for a rough comparison, the fluorescent protein mCherry, whose spectrum is also included in the figure, has a similar size profile of around 4 nm. Previous results suggested that the same carbon dots could be used for fluorescence imaging at different colors, though not with the same sensitivities. Further investigations targeting color variations of bright fluorescence emissions over the visible spectrum, such as the exploration of other carbon nanoparticle surface passivation schemes, while maintaining the overall size profiles of the carbon dots are warranted, thus to take full advantage of their different fluorescence emission mechanism from that in conventional semiconductor QDs.

The development of ultracompact fluorescence probes based on carbon dots offers significant values both fundamentally and technologically. On the fundamental side, the experimental confirmation on the preparation of the very small carbon dots of multiple fluorescence colors in the visible spectrum serves to support the existing mechanistic framework for carbon dots, namely, that the fluorescence emissions are due to the radiative recombination of the trapped or confined electrons and holes from the initial charge separation following the photoexcitation. The emission colors may be affected by changes in the core carbon nanoparticle size, with associated changes in the particle surface properties such as the surface curvature,
Experimental Section

Materials. Carbon nanopowder (<50 nm, purity 99+%), 13C powders (isotopic purity 99%), and 2,2'-ethylene(diamino)bisis-(ethylenamine) were purchased from Sigma-Aldrich, and fine-extruded graphite rods (carbon content 99+%%) from Graphistore, Inc. Thiophene chloride (≥99%) was obtained from Alfa Aesar, nitric acid from VWR, and Sephadex G-100 gel from GE Healthcare. Dialysis membrane tubing (cutoff molecular weight 500) was supplied by Spectrum Laboratories. Water was deionized and purified by being passed through a Labconco WaterPros water purification system.

Measurements. Optical absorption spectra were recorded on a Shimadzu UV2501-PC spectrophotometer. Fluorescence spectra were measured on a Jobin-Yvon emission spectrometer equipped with a 450 W xenon source, Gemini-180 excitation and emission monochromators, and a photon-counting detector (Hamamatsu R928P PMT at 950 V). The nonlinear instrumental responses at both excitation and emission sides of the spectrometer were corrected by using separately determined correction factors with respect to different excitation and emission wavelengths.12 Raman spectra were obtained on a Jobin-Yvon T64000 Raman spectrometer equipped with a Melles Griot He–Ne laser (35 mW) for 632.8 nm excitation, a triple monochromator, a liquid-nitrogen-cooled symmetry detector, and an Olympus BX-41 microscope for sampling. FT-IR spectra were collected on a Thermo Nicolet Nexus 670 FT-IR/NIR spectrometer, with the samples for analysis deposited on the surface of a KBr crystal pellet. NMR measurements were performed on a Bruker Avance 500 NMR spectrometer. Atomic force microscopy images were acquired in the acoustic ac mode on a Molecular Imaging PicoPlus AFM system equipped with a multipurpose scanner and a NanoWorld point probe NCH sensor. The height profile analysis was assisted by using the SPIP software distributed by Image Metrology. Transmission electron microscopy images were obtained on a Hitachi H9500 TEM system.

13C-Enriched Carbon Nanoparticles. The carbon soot containing the nanoparticles was obtained in the arc-discharge production, as reported previously.39 A graphite rod was made hollow and then filled with a mixture of the commercially supplied 13C powders and graphite cement. The discharge chamber was purged with helium and then stabilized at 1 atm (101.325 kPa) in a helium atmosphere. The rods were vaporized with a direct current of 70 A (28 V). The as-produced carbon soot was collected and dispersed in dimethyloctamomamide (DMF) with ultrasonication (Crest Ultrasonics, model 950 DA, 50–60 Hz) for 24 h. The DMF was removed by evaporation, and the carbon particles were recovered. The 13C content in the sample was estimated in terms of Raman spectral shifts, as validated previously.31

EDA-Carbon Dots. The precursor carbon nanoparticles were refluxed in an aqueous nitric acid solution (2.6 M) for 12 h, dialyzed against fresh water, and then centrifuged at 1000 g to retain the supernatant. The recovered sample was refluxed in neat thionyl chloride for 12 h. Upon the removal of excess thionyl chloride, the sample (50 mg) was mixed well with carefully dried EDA liquid (600 mg) in a round-bottom flask, heated to 120 °C, and vigorously stirred under nitrogen protection for 3 days. The reaction mixture back at room temperature was dispersed in water and then centrifuged at 20800 g to retain the supernatant. The solution was filtered through a Sephadex G-100 column (packed in house with commercially supplied gel sample).10,46 and the colored section with high fluorescence quantum yields was collected, followed by dialysis against fresh water (dialysis tubing cutoff molecular weight 500) to yield an aqueous solution of the EDA-carbon dots.11 The 13C-enriched EDA-Carbon dots were prepared from the 13C-enriched precursor carbon nanoparticles by following the same experimental procedures.11

Conclusion

The reported work demonstrated that small PEG diamine molecules could be used to functionalize carbon nanoparticles to produce surface-passivated carbon dots with optical properties similar to those of the more extensively studied PEG1500–carbon dots. The results suggest generally excellent solubility characteristics of carbon dots, as the passivation agent layer in the overall structure of the EDA-carbon dots is relatively small. Smaller functionalization molecules are expected to be in favor of smaller carbon nanoparticles in the solubilization, resulting in more compact carbon dots. In fact, the EDA-carbon dots were found to be comparable in size to GFPs, with expanded application potential in bioimaging and beyond. The demonstration on the reduction in probe size without sacrificing the intrinsic properties of carbon dots may stimulate further investigations on the preparation of bright fluorescent probes of even smaller footprints.
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REFERENCES AND NOTES


Carbon-based quantum dots for fluorescence imaging of cells and tissues

Pengju G. Luo,a Fan Yang,a Sheng-Tao Yang,*b Sumit K. Sonkar,a Liju Yang,c Jessica J. Broglie,c Yun Liu a and Ya-Ping Sun*a

Carbon dots (or carbon quantum dots in some literature reports), generally small carbon nanoparticles with various surface passivation effects, have attracted widespread attention in recent years, with a rapidly increasing number of research publications. The reported studies covered many aspects of carbon dots, from the development of many new synthetic methodologies to an improved mechanistic elucidation and to the exploration of application opportunities, especially for those in the fluorescence imaging of cells and tissues. There have also been significant advances in the establishment of a shared mechanistic framework for carbon dots and other carbon-based quantum dots, graphene quantum dots in particular. In this article, representative recent studies for more efficient syntheses of better-performing carbon dots are highlighted along with results from explorations of their various bioimaging applications in vitro and in vivo. Similar fluorescence properties and potential imaging uses of some graphene quantum dots are also discussed, toward a more consistent and uniform understanding of phenomenologically different carbon-based quantum dots.

1. Introduction

Semiconductor nanocrystals of physical dimensions smaller than the exciton Bohr radius are commonly referred to as quantum dots (QDs) for the quantum confinement effect,

manifested in terms of systematically predictable dependence of optical properties, especially fluorescence emission colors, on the nanocrystal dimension.1-3 More specifically, conventional semiconductor QDs have been widely pursued as superior fluorescence dyes for imaging and other biomedical applications.4-7 In fact, the rationale for the use of QDs over organic dyes is now generally accepted in the literature.8 Similarly bright and colorful fluorescence emissions have been found in other nanomaterials containing no conventional semiconductors, and those fluorescent nanomaterials are often considered, more phenomenologically perhaps, as QDs as well, despite the absence of any classical quantum confinement

Pengju G. Luo has been a faculty member at Sherman College of Chiropractic since 2006. He received his medical degree in Clinical Medicine from Tongji Medical University, Wuhan, China in 1997 and his Ph.D. in Microbiology from Clemson University in 2006. His current research interests are in the interdisciplinary areas of nanotechnology and biological and biomedical sciences, focusing on bioapplications of various nanomaterials such as nanoparticles, nanotubes, and carbon dots.

Fan Yang obtained his B.S. degree in Chemistry from Zhejiang University, China in 2010. He is currently pursuing his Ph.D. at Clemson University under the supervision of Prof. Ya-Ping Sun. His research focus is on the synthesis of carbon dots for their applications in bioimaging and energy conversions.
effect in most cases. Among more popular and promising recent additions to the loosely defined QD family are carbon-based QDs.\textsuperscript{7–12} Unlike conventional semiconductor QDs, whose typical contents of heavy metal elements such as cadmium or lead have generated much concerns with respect to \textit{in vivo} or \textit{in vitro} uses,\textsuperscript{7,13–15} carbon-based QDs are pursued as nontoxic alternatives in the development of fluorescence imaging agents for various biomedical purposes.\textsuperscript{10–12}

Carbon-based QDs generally refer to brightly fluorescent carbon dots (Scheme 1, Fig. 1)\textsuperscript{9} and graphene QDs (GQDs).\textsuperscript{16,17} The former is based solely on defects in the carbon nanostructures,\textsuperscript{9–11,18,19} while the latter is mechanistically divided into two categories, with one depending on defects and the other on isolated $\pi$ domains on graphene sheets.\textsuperscript{20} However, since the defect-derived fluorescence emissions in GQDs are more dominating, a strong case has been made that a significant portion of the observed fluorescence properties in GQDs may be understood in terms of the same mechanistic framework developed for carbon dots.\textsuperscript{21} Therefore, in this article we will focus on highlighting studies of carbon dots and their mechanistically similar GQDs as fluorescence bioimaging agents that are performance-wise competitive to those of conventional semiconductor QDs. The existing uses of these carbon nanomaterials in fluorescence imaging of cells and tissues are also highlighted, and issues and prospects in such applications discussed.

2. Carbon dots

Carbon dots (also referred to as carbon quantum dots or C-Dots) are generally small carbon nanoparticles with various surface passivation via modification or functionalization.\textsuperscript{9–12,12,21} Ever since the original report on carbon dots in 2006,\textsuperscript{9} there have been extensive investigations by many research groups around the world, mostly driven by the need to develop biomarkers and bioimaging agents. Since the original report on carbon dots in 2006,\textsuperscript{9} there have been extensive investigations by many research groups around the world, mostly driven by the need to develop biomarkers and bioimaging agents.

Sheng-Tao Yang received his B.Sc. in 2006 and Ph.D. in 2011 at Peking University majoring in chemistry. During 2008–2009, he studied as a visiting student under Prof. Ya-Ping Sun’s supervision in the Department of Chemistry at Clemson University. Since 2011, he works as a lecturer at College of Chemistry and Environment Protection Engineering, Southwest University for Nationalities. His current research focuses on the bioapplications and biosafety of carbon nanomaterials.

Dr Liju Yang is currently an associate professor in the Department of Pharmaceutical Sciences and the Bio-manufacturing Research Institute and Technology Enterprise (BRITE) at North Carolina Central University (NCCU). She received her B.S. degree in Chemistry from Hangzhou Teachers College and M.S. degree in Analytical Chemistry from Hangzhou University, China, in 1991 and 1996, respectively. She received her Ph.D. degree in Biological and Agricultural Engineering from the University of Arkansas in Dec 2003, and did her postdoctoral research in the School of Electrical and Computer Engineering at Purdue University. She joined NCCU as a faculty member in Feb 2006. Her current research focuses on two areas: (i) the development of novel biosensors, biochips and microdevices for applications in food safety, drug discovery, and biomedical diagnostics; and (ii) nanomaterials interfacing with biological entities.

Sumit K. Sonkar received his Ph.D. in chemistry from the Indian Institute of Technology Kanpur, India in 2012. He was a postdoctoral scientist in Prof. Ya-Ping Sun’s group at Clemson focusing on the development and characterization of carbon-based quantum dots for bioimaging and energy related applications.

Ya-Ping Sun earned his Ph.D. at Florida State University in 1989. After postdoctoral training at University of Texas at Austin, he joined the Clemson faculty in 1992. He has been the Frank Henry Leslie Chair Professor of Natural & Physical Sciences since 2003. His research interests are primarily in carbon-based nanomaterials.
Review

Scheme 1  Left: a cartoon illustration on the simple amino-PEG-functionalized carbon dots, where the colored corona-like layer illustrates the origin of fluorescence emissions. Right: the presently more accepted mechanistic framework on surface-confined electrons and holes and their recombinations being responsible for the fluorescence emissions in carbon dots (which could be quenched by either electron acceptors or donors, as found experimentally).

Fig. 1  Aqueous solutions of the as-synthesized carbon dots excited at the indicated wavelengths in nm and photographed directly (top), and excited at 400 nm and photographed through band-pass filters of different wavelengths as indicated (bottom) (from ref. 9).

the world, with major progress made in the development of synthetic strategies,9,24 in the structural and mechanistic understandings of carbon dots,9 and in the exploration of their potential applications.25,26

The original finding of carbon dots was inspired by the results from studies of functionalized carbon nanotubes.9,10 For both single-walled and multiple-walled carbon nanotubes, the passivation of surface defects with the chemical functionalization resulted in bright and colorful fluorescence emissions at various excitation wavelengths from UV to near-IR.27–29 The inspiration was associated with the recognition that small carbon nanoparticles are full of surface defects, which upon effective surface passivation via chemical functionalization should be equally or more fluorescent over similar spectral regions. Indeed, it was found that the solutions of surface-functionalized small carbon nanoparticles, now called carbon dots (Scheme 1), exhibited bright and colorful fluorescence emissions over the visible and extending into the near-IR (Fig. 1).9,22,21 Carbon dots were also found as being strongly multiphoton fluorescent, with extremely large two-photon cross-sections observed in the pulsed laser excitation (800–900 nm) of carbon dots for visible fluorescence emissions.9 With their bright one- and multi-photon fluorescence emissions and other linear and nonlinear optical properties,9,21 carbon dots have been widely pursued for a variety of interesting and/or unique applications, with examples including bioimaging,32 photodynamic therapy,33 fluorescent probing and analysis,34 and photocatalysis.35 Since carbon dots are generally nontoxic (no more toxic than the selected surface functionalization molecules),36 and also generally high in photostability,9,22 their uses in fluorescence imaging of cells and tissues may prove to be particularly significant. For all of these potential applications, much effort has been made in the development of synthetic methodologies for the targeted properties and uses, which are highlighted as follows.

Synthesis

Since 2006,9 research groups worldwide have developed many physical and chemical methods for the production of carbon nanoparticles, which were used as precursors for carbon dots or suspended in various solvents for fluorescence measurements directly. Among more popular methods were laser ablation,9 electrochemical oxidation,37 chemical oxidation,38 hydrothermal carbonization,39 and pyrolysis.40 In a broad definition, carbon dots may include “naked” carbon nanoparticles in aqueous and other suspensions, in which multicolor fluorescence emissions under different excitation conditions have been reported by a number of research groups,9 though the observed emission quantum yields were generally low to very low for the truly naked.42–47 It was argued that even for the truly naked carbon nanoparticles in various suspensions, the solvent molecules were likely responsible for some limited yet meaningful particle surface passivation, thus somewhat brighter emissions in those solvents that such passivation effects were more significant.45 Therefore, generally speaking brighter fluorescence emissions were observed in carbon dots with the core carbon nanoparticles surface-passivated, either deliberately or by default from some synthesis methods.

The original report on carbon dots was on functionalized small carbon nanoparticles, namely that the particle surface passivation was deliberate in terms of chemical functionalization.9 Experimentally, the precursor carbon nanoparticles were treated with nitric acid for the introduction of surface groups subsequently used for the functionalization chemistry. The functionalization agents included the now widely used oligomeric polyethylene glycol diamine (PEG1500N) and amino-polymer (PPEI-EI), and the resulting carbon dots exhibited
bright fluorescence emissions of quantum yields up to 10% or higher. Since then, various precursor carbon nanoparticles were functionalized for brightly fluorescent carbon dots, including commercially available carbon nanopowders and carbon nanoparticles separated from candle soot, gas soot, lampblack, and other carbon nanomaterials.

For the surface functionalization, a long list of molecules and species have been investigated, such as PEGs of different molecular weights, mercaptosuccinic acid, ethylenediamine, polyethylenimine (PEI), and oleylamine. It is probably no coincident that most of these surface passivation agents are molecules with amino moieties, which must be particularly effective in the desired passivation for brighter fluorescence emissions.

A more popular approach has been to combine the formation of precursor carbon nanoparticles with surface passivation, namely “one-pot” syntheses or their equivalents. These syntheses were sometimes misunderstood as being capable of producing brightly fluorescent carbon dots without surface passivation, though more likely the syntheses had the passivation effects built in without any separate procedures. For example, graphite and carbon nanotubes were electrochemically oxidized into carbon dots, in the presence of specific surface functional groups. More popular than electrochemical synthesis was the use of hydrothermal treatments, which were demonstrated as being effective in converting organic molecules into fluorescent carbon dots. Among organic precursors in the reported studies were glucose, EDTA, citric acid, thiomalic acid (TMA), 1,3,5-trimethylbenzene (TMB), or even fullerene and somewhat unusual yet perfectly normal precursors such as orange juice. In one interesting study of fullerene-based carbon dots, fullerene was first conjugated with tetraethylene glycol (TEG), and the TEG–fullerene conjugates were aggregated into larger nanoparticles at higher concentrations (Fig. 2). The observed fluorescence color could be regulated by the conjugate concentration, with higher concentration red-shifting the fluorescence emission. However, the observed fluorescence quantum yields were all relatively low, less than 1%.

In addition to the popular hydrothermal syntheses, conceptually similar sonication and microwave irradiation techniques were also employed for the purpose. In all these methods, the carbonization was likely responsible for the formation of carbon nanoparticles, with the passivation effect due to the residual organic species in the resulting carbon dots. Nevertheless, while the fluorescence quantum yields of the carbon dots from these one-pot syntheses were significant, they were still generally lower than those in carbon dots prepared from the deliberate surface functionalization of precursor carbon nanoparticles.

Yang et al. added templating effect to the synthesis of carbon dots from organic precursors, dubbed “soft-hard template approach,” to manipulate the dot sizes, compositions, crystallinities and fluorescence properties. The soft template was the copolymer Pluronic P123, and the hard template was the ordered mesoporous silica (OMS) SBA-15, with the organic precursors including 1,3,5-trimethylbenzene (TMB), diaminobenzene (DAB), pyrene (PY), and phenanthroline (PHA).

**Greener syntheses**

There has been much recent effort on developing greener production methods for carbon dots, valuable to the preparation for larger scale applications. A major feature in the reported productions was the use of biological species or natural products as precursors, including especially peptides and proteins and a variety of carbohydrates. Examples for proteins were meat products, natural silk, bovine serum albumin (BSA), egg shell membrane ash, hair fiber and gelatins, and those for carbohydrates included glycerol, chitosan, cellulose and cyclodextrin, and sucrose. In addition to the orange juice discussed above, other food products or byproducts used as precursors for carbon dots were pretty diverse, including other fruit juices, bread, caramels, honey, and sweet pepper. Even greener precursors were those from biomass and bio-wastes, such as grass, watermelon peel, plant leaves, plant bark (willow), and paper ash. Some representative studies are highlighted as follows.

Wang et al. used the char from overcooked BBQ meat as precursor for carbon dots, with a purpose to demonstrate the versatility with respect to the dots in terms of their structures, composition, and properties (Fig. 3). The resulting carbon dots with PEG1500 for surface functionalization exhibited fluorescence quantum yields up to 40%. Although the expectation was for the carbon nanoparticles to be primarily from the carbonization of meat proteins, their chemical compositions were likely more complicated. Therefore, the high fluorescence performance of the carbon dots suggested that carbon dots are intrinsically versatile in the core structure and composition and performance-wise robust.

Bhunia et al. synthesized carbon dots less than 10 nm in diameter from the carbonization of carbohydrates. There were
two versions of the dots, hydrophobic and hydrophilic, both of which exhibited blue and green fluorescence emissions. The hydrophobic ones were produced by mixing different amounts of the carbohydrate with octadecylamine and octadecene for being heated up to 300 °C for up to 30 min. The hydrophilic ones were synthesized by heating aqueous solution of the carbohydrate at different pH values, including the use of concentrated sulphuric acid. The hydrophilic dots with yellow and red emissions were synthesized by mixing an aqueous solution of the carbohydrate with concentrated phosphoric acid and then heating at 80–90 °C for up to 60 min (Fig. 4).44

Wu et al. synthesized carbon dots from honey.87 The fluorescence emissions were enhanced by the surface passivation with hyperbranched polymers, with the enhancement was also observed in the near-IR spectral region. De et al. reported the synthesis of small carbon dots from banana juice, for which the procedure was simply heating the banana (Musa acuminate) juice at 150 °C for 4 h.84

Krysmann et al. demonstrated that carbon dots could be produced from soft tissue biomass.89 The pyrolytic decomposition of shredded grass at 300 °C in air resulted in the formation of well-defined carbogenic nanoparticles, which were further functionalized with amino molecules (ethanolamine, 2-aminoethane sodium sulfonate, and oleylamine) to yield hydrophilic and hydrophobic carbon dots. As reported, these carbon dots were particularly stable.89

**Property manipulation and enhancement**

There have been many reports on strategies aimed at manipulating the structural features and optical properties of carbon dots for improvements, especially with respect to increasing the fluorescence brightness and extending the emissions into red and near-IR special regions for enhanced bioimaging applications. Apparently higher fluorescence quantum yields in carbon dots could be achieved through more effective surface passivation of the core carbon nanoparticles95 and doping in the core96 and on the surface.22,97

Carbon dots with deliberate surface passivation via chemical functionalization are generally more fluorescent.22 The samples as-prepared are typically mixtures of carbon dots with different fluorescence quantum yields, attributed to variations in the surface functionalization, which could be recognized in chromatography columns to enable fractionations for the harvesting of the highly fluorescent carbon dots.95 Wang et al. first applied a conventional size-exclusion gel column to the fractionation to obtain PEG1500N-functionalized carbon dots of fluorescence quantum yields 60% or higher, which were shown to be performance-wise competitive to the commercially supplied CdSe–ZnS QDs in the comparable spectral region.95 The fractionation approach has since been extended to include separation techniques such as selective centrifugation, HPLC, and capillary zone electrophoresis for harvesting carbon dots of better performance.98–100 For example, in the capillary zone electrophoresis, the negatively charged fraction was found to be lower in fluorescence quantum yield but higher in optical absorptivity, whereas positively charged and neutral fractions were of higher fluorescence quantum yields.100

Altering the chemical structure and/or composition in carbon dots has been a promising approach for property enhancements. In an early study, Sun et al. doped the core carbon nanoparticle surface with inorganic salts (ZnO, ZnS, or TiO2) before the chemical functionalization.22 The doped carbon dots (denoted as C2ZnO-Dots, C2ZnS-Dots, or C2TiO2-Dots) exhibited much brighter fluorescence emissions than those of the dots without any doping. It was argued that the inorganic salts must have augmented the passivation effect when combined with the surface functionalization by organic molecules.22 The as-synthesized samples of the doped carbon dots were apparently mixtures as well, containing dots of close to quantitative fluorescence emissions. In a follow-up study, Anilkumar et al. fractionated the as-prepared sample of C2ZnS-Dots on a gel column to harvest carbon dots of observed fluorescence quantum yields more than 75% in the green, the spectral region matching well with that covered by green fluorescence proteins.97
Various other doping strategies have been explored.\textsuperscript{65,77,96,101} For example, Dong \textit{et al.} prepared nitrogen- and sulfur doped carbon dots for high fluorescence quantum yields.\textsuperscript{96} The hydrothermal synthesis involved the use of citric acid and L-cysteine as precursors. Chandra \textit{et al.} synthesized sulfur-doped carbon dots from TMA through pyrolysis in the presence of concentrated H\textsubscript{2}SO\textsubscript{4} and then the isolation and polymerization with excess NH\textsubscript{4}OH.\textsuperscript{65,77} Jahan \textit{et al.} reported B/N co-doped carbon dots with deliberate surface passivation for high fluorescence yields. The synthesis was based on the hydrothermal processing of boric acid and N-(4-hydroxyphenyl)glycine in water.\textsuperscript{101}

As reported originally,\textsuperscript{9} carbon dots are multicolor in fluorescence emissions. However, the available carbon dots are generally more fluorescent in the green and shorter wavelength regions, which are less desirable for their uses as bioimaging agents.\textsuperscript{12} Therefore, there has been significant recent effort on manipulating carbon dots for enhanced red and near-IR fluorescence emissions.\textsuperscript{94,102,103} For example, Bhunia \textit{et al.} reported the hydrothermal synthesis of carbon dots from carbohydrate precursor for enhanced red fluorescence.\textsuperscript{94} Somewhat unusual, however, was the use of UV excitation for red emissions.

Huang \textit{et al.} reported a different strategy to achieve longer-wavelength fluorescence emissions by attaching dyes of known red/near-IR fluorescence to carbon dots.\textsuperscript{102} The selected dye was ZW800 (fluorescent in the near-IR), and it was conjugated to PEG\textsubscript{1500}N-functionalized carbon dots in terms of the classical coupling chemistry. The ZW800-conjugated carbon dots exhibited bright fluorescence emissions in green and red/near-IR spectral regions. Similarly, Ce6 (chlorin e6)-conjugated carbon dots were prepared, in which the Förster resonance energy transfer (FRET) allowed the excitation at 430 nm to result in red fluorescence emissions (Fig. 5).\textsuperscript{103}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Figure4.pdf}
\caption{Digital images on gram-scale solid samples of fluorescent carbon nanoparticles (FCN), solutions under various excitations, and corresponding absorption (solid line left), excitation (dotted line), and emission (colored solid line right) spectra. Emission spectra were measured with excitation at 370 nm for FCN\textsubscript{blue}, 400 nm for FCN\textsubscript{green}, 425 nm for FCN\textsubscript{yellow}, and 385 nm for FCN\textsubscript{red}. All excitation spectra were recorded by monitoring at the respective emission maxima (from ref. 94).}
\end{figure}
3. Fluorescence imaging of cells and tissues

There have been a number of studies on the use of carbon dots for fluorescence imaging of cells and tissues, from straightforward cell imaging to specific tissue targeting, and to theranostics for concurrent effects of bioimaging, drug release, and/or photodynamic therapy. Explorations on the in vivo imaging with various carbon dots have also been reported by several research groups.

Cellular uptake and fluorescence imaging

Carbon dots in most configurations are readily taken by cells, enabling fluorescence imaging of cells with both one- and multiple-photon excitations, as demonstrated in early studies of carbon dots. For more recent examples, Zhang et al. prepared dots from polydopamine (PDA-FONs) for cell imaging. At 405 nm and 458 nm excitations, bright green and yellow fluorescence emissions, respectively, were observed in the cytoplasm, but not in the cell nucleus. Yan et al. used carbon dots from cellulose and cyclodextrin via hydrothermal synthesis for cell imaging. Upon incubation with mouse melanoma cells (B16-F10) for 5 h, the carbon dots were taken up by the cells with the observation of meaningful blue, yellow, and red fluorescence emissions corresponding to different excitation wavelengths, though the imaging resolution was not high enough for a more precise determination of the locations for the dots. Zhang et al. reported the synthesis of aqueous compatible carbon dots in one-pot hydrothermal processing of nanodiamond. The carbon dots were internalized into the NIH-3T3 cells upon incubation, with green and yellow fluorescence emissions at 405 nm and 458 nm excitations, respectively, found in the cell cytoplasm. Wei et al. prepared carbon dots from paper ash and demonstrated that these dots could be efficiently taken up by human L02 hepatic cells, with rather low cytotoxicity.

In some imaging experiments, carbon dots were also found in the cell membranes. For example, Chen et al. prepared carbon dots by carbonizing sucrose with oil acid for imaging 16HBE cells. Green fluorescence emissions were observed around the cell membrane, in addition to the cytoplasm, though only much weaker fluorescence was detected in the cell nucleus. Zhang et al. used one-pot hydrothermal synthesis with bovine serum albumin (BSA) as the carbon source and 4,7,10-trioxa-1,13-tridecanediamine (TTDDA) as the surface passivation agent to prepare carbon dots for imaging colorectal carcinoma cells (SW1116). After incubation for only 3 h, the carbon dots were internalized into the cells, exhibiting blue fluorescence emissions around the cell membrane and cytoplasm region under UV excitation. Similarly, Ding et al. applied the hydrothermal synthesis to the preparation of TTDDA-passivated carbon dots. These dots were used to label HeLa cells, exhibiting low toxicity effect on the cells even at high concentrations of up to 5 mg mL\(^{-1}\).

Carbon dots from natural products or more biocompatible precursors were also evaluated in cell imaging experiments. For example, Sahu et al. used carbon dots from orange juice in the imaging of MG-63 cells. Blue and green fluorescence emissions at 405 nm and 488 nm excitations, respectively, were observed in the cell cytoplasm, but no fluorescence found in cell nuclei. Sachdev et al. applied microwave-assisted pyrolysis to the one-step synthesis of PEG-4000 passivated carbon dots from chitosan. The carbon dots were evaluated for their potential
bio-labeling uses in reference to \textit{S. aureus} and recombinant green fluorescent protein (GFP)-expressing \textit{E. coli} as model systems.\textsuperscript{83} Saxena \textit{et al.} isolated water-soluble carbon dots from charred bread for the fluorescence imaging of human red blood cells.\textsuperscript{83} The carbon dots were found to be relatively nontoxic to the human erythrocytes, and even exhibited higher fluorescence quantum yields in the presence of the blood plasma, which was rationalized as being due to interactions of biomolecules in the blood plasma with the carbon dots.\textsuperscript{83} Saxena \textit{et al.} also synthesized carbon dots from wood wool for fluorescence imaging over the full life cycle of different species of mosquitoes (\textit{Anopheles} sp., \textit{Aedes} sp. and \textit{Culex} sp. larvae). The carbon dots at higher concentrations could have growth restriction effects, slowing down the metabolism in the larval stage of mosquitoes, thus preventing their reaching the pupae stage to eventually lead to the death of the mosquitoes (Fig. 6).\textsuperscript{107}

Several reports highlighted the use of doped carbon dots for cell imaging. Xu \textit{et al.} used N-doped carbon dots for the fluorescence labeling of HeLa cells and HepG2 cells. Multicolor fluorescence emissions corresponding to different excitation wavelengths were observed in the cell cytoplasm.\textsuperscript{108} Chandra \textit{et al.} prepared sulphur-doped carbon dots from TMA. These dots could be readily dispersed in water, with high photostability. In the imaging of \textit{E. coli} cells, blue fluorescence emissions were observed at 405 nm excitation. The negatively charged oxygen and sulfur groups in the doped carbon dots could apparently bind with positively charged DNA–PEI complexes, resulting in bright fluorescence.\textsuperscript{83} Wu \textit{et al.} synthesized nitrogen-doped amphoteric carbon dots from natural silk (from \textit{Bombyx mori}, as \textit{Bombyx mori} silk contains a high percentage of nitrogen. The doped carbon dots were amphoteric, depending on the solution pH, and stable in resisting relatively harsh chemical environments. The use of these dots as probes for the fluorescence imaging of A549 cells was evaluated.\textsuperscript{73} A549 cells were also used in the study by Qu \textit{et al.}, with carbon dots from dopamine as precursor in the hydrothermal synthesis.\textsuperscript{109} Beyond cell imaging, the carbon dots were employed in the determination of Fe\textsuperscript{3+} ions in water samples and dopamine in human urine and serum samples, taking advantage of the distinctive catechol groups on the carbon nanoparticle surface.

\textbf{With specific targeting}

Han \textit{et al.} used polyethyleneimine-modified carbon dots to examine the fluorescence labeling of HeLa cells. The carbon dots conjugated with the CEA8 antibody could label HeLa cells upon incubation for only 90 min, and the labeling was visualized by the green fluorescence contour of the cell shape. In the control experiment without the CEA8 antibody, carbon dots were not found under UV excitation.\textsuperscript{110}

Bhunia \textit{et al.} applied the carbon dots from the carbonization of carbohydrates to the fluorescence imaging of HeLa cells.\textsuperscript{94} However, the dots had very low non-specific binding to cells, probably due to their small hydrodynamic sizes and low surface charge. Upon the dots being linked to TAT peptide or folate, their cellular uptake was significantly enhanced, with fluorescence emissions readily detected under a confocal microscope.\textsuperscript{94}

Lee \textit{et al.} prepared carbon dots with the maleimide-terminated TTA1 aptamer (targeting tenascin C proteins (Tnc) proteins) for the fluorescence imaging of cancer cells.\textsuperscript{111} Since Tnc proteins are highly expressed in HeLa cells and C6 cells, but rarely expressed in CHO cells, the TTA1–carbon dots were found to be significantly selective to HeLa cells and C6 cells, with only minor up-taking by CHO cells.\textsuperscript{111}

\textbf{Bioanalyses}

Salinas-Castillo \textit{et al.} prepared carbon dots \textit{via} the pyrolysis of citric acid in the presence of PEI.\textsuperscript{112} The carbon dots were highly selective to Cu\textsuperscript{2+}, and could readily be swallowed by NIH-3T3 cells. At 850 nm excitation the observed up-conversion fluorescence in the cells could be quenched partially with the addition of Cu\textsuperscript{2+}, suggesting potential applications of these carbon dots in the intracellular detection of the copper cation.\textsuperscript{112}

Kong \textit{et al.} used carbon dots for two-photon fluorescence imaging and biosensing.\textsuperscript{113} In the experiments the proton responsive molecule 4-(aminomethylphenyl)-2,2':6',2''-terpyridine (AE-TPY) was linked to carbon dots to be used as sensors for monitoring pH variations. At a lower pH, AE-TPY could bind

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image6.png}
\caption{Growing larvae treated with 3.0 mg L\textsuperscript{-1} water-soluble carbon nanoparticles. The first row represents the auto fluorescence of the control mosquito larvae. The other six rows (from top to bottom) are fluorescence images on the indicated days for treated larvae of \textit{Culex} sp. Left, middle and right column images are those observed under 488 nm, 561 nm and the merged image of 488/561 nm band pass filters, respectively (reproduced from ref. 107).}
\end{figure}
with proton, resulting in stronger two-photon fluorescence in the carbon dots. Upon the incubation of the conjugates with A549 cells, fluorescence emissions from the carbon dots could be detected in the perinuclear regions of the cells. With the addition of choline CH$_3$SO$_3$-Ringer’s solution to increase cellular cytosolic Na$^+$, the intracellular pH decreased, accompanied by significantly increased fluorescence intensities. Then, the addition of 100 mM Na$^+$ led to increased pH, thus lower fluorescence intensities. The utility of carbon dots as pH sensors was also demonstrated in A549 cancer cell tissue. The fluorescence intensities sharply increased when the pH was decreased from 7.8 to 6.4. In addition, the 3D two-photon fluorescence imaging of carbon dots was used to reveal the pH gradients over the depth of 65–185 μm in tissue. 112

Shen et al. also used pH-sensitive carbon dots for cell imaging. Upon incubation with HeLa cells, the carbon dots could readily penetrate the cell membrane, with low cytotoxicity and favorable biocompatibility to stain the whole cells. 113

Theranostics

There have been a number of reports on using carbon dots for theranostics to achieve the concurrent effects of bioimaging, drug release, photodynamic therapy, antimicrobial, and/or transfection. 12 For example, Lai et al. prepared carbon dots from glycerol inside mesoporous silica nanoparticles for both fluorescence imaging and drug release. 79 The carbon dots–SiO$_2$ nanoparticles were further capped with PEG molecules to enhance the fluorescence brightness, stability, and biocompatibility. These PEGylated carbon dots–SiO$_2$ nanoparticles were able to deliver the anticancer drug doxorubicin (DOX) into HeLa cells, exhibiting improved efficacy than that of free DOX. The fluorescence imaging was performed at different incubation times for the nanoparticles with HeLa cells. At 2 h incubation, most DOX was co-localized with carbon dots, as indicated by the purple fluorescence (a mixture of red from DOX and green from carbon dots); and at 24 h, red fluorescence due to DOX was found in the cell nuclei, while green fluorescence from carbon dots remained in the cytoplasm. The release of DOX was more pronounced post 48 h incubation. 79

In a conceptually similar approach, He et al. encapsulated carbon dots and Si nanoparticles in the mesoporous SiO$_2$ for nanocomposites, denoted as C@Si–SiO$_2$, to be used in fluorescence imaging and drug delivery. 114 The C@Si–SiO$_2$ was coated with PEG molecules and linked with targeting moieties hyaluronic acid for enhanced selectivity in the drug delivery. The resulting C@Si–SiO$_2$ conjugate carrying the drug camptothecin (CPT) in MDA-MB-468 cells exhibited yellow fluorescence emissions in the cell cytoplasm. The blue fluorescence due to CPT was found to be shifted from the yellow fluorescence spots,

![Fig. 7](image_url)

**Fig. 7**  Cellular imaging of carbon dots–PEI/Au–PEI/pDNA assembly in HeLa cells. (a) Fluorescence of carbon dots–PEI (blue), (b) TOTO-iodide labeled pDNA (red), and (c) merged image at different time interval (from ref. 116).
Kim et al. coupled carbon dots with gold nanoparticles for an assembly, which was then conjugated with PEI-pDNA for delivering DNA to cells. Fluorescence emissions from the assembly of carbon dots–gold nanoparticles could be quenched by pDNA, so that the release of pDNA could be probed by the recovery of fluorescence signals. The experimental results suggested that the assembly did enter the cells, with the carbon dots located in the cell cytoplasm and the released pDNA entering the cell nuclei, achieving significant transfection efficiency (Fig. 7). Pandey et al. used carbon dots to functionalize gold nanorod for the delivery of doxorubicin in a multi-modality fashion, including the drug delivery, photothermal therapy, and bioimaging on the same platform.

Fluorescence imaging in vivo

Carbon dots were demonstrated in earlier studies as being applicable to fluorescence imaging in vivo. Recent efforts have confirmed or extended the application potential. For example, Wu et al. used carbon dots from honey for in vivo imaging experiments in a mouse model, from which the results showed high contrast enhancement in auxiliary lymph node. As pointed by the authors, the rapid lymphatic transport of these dots might be a valuable property, offering greater convenience and reduced procedural expense, as well as improved surgical advantage as the subject is positioned on the table for easier resection.

Li et al. investigated the cellular uptake and biodistribution of carbon dots for optical imaging. The co-localization of carbon dots (blue fluorescence) and fluorescent markers (red fluorescence) was used to probe various cellular organelles, including lysosome/endosome, Golgi body, mitochondria, and endoplasmic reticulum. The results suggested that the carbon dots were largely trapped in lysosome/endosome, though their presence in Golgi body, mitochondria, and endoplasmic reticulum was also observed. For the imaging in vivo, the carbon dots were introduced into mice through intravenous injection. Organs were harvested and sliced for fluorescence imaging at 500 nm emission with 405 nm excitation. Blue fluorescence was observed in heart, liver, spleen, kidneys, lungs, brain, and small intestine, with the brightest being in the spleen sample.

Srivastava et al. fabricated iron oxide-doped carbogenic nanocomposite (IO–CNC) for multi-modality (magnetic resonance (MR)/fluorescence) bioimaging. The IO–CNC preparation was the thermal decomposition of organic precursors in the presence of small Fe3O4 nanoparticles (average size 6 nm). The IO–CNC could be taken by RAW264.7 cells, and the fluorescence was mainly detected in the cell cytoplasm. For the imaging in vivo, the IO–CNC was introduced into rats through intravenous injection. Fluorescence signals due to the IO–CNC were observed in the spleen slide samples. The MRI imaging results suggested enhanced signals in the brain blood vessel under both T1 and T2 models (Fig. 8).

Huang et al. extended the spectral range of carbon dots to red/near-IR by attaching the fluorescence dye ZW800 to the

with some blue CPT reached the cell nucleus, suggesting that CPT was released from the C@Si–SiO2 carrier. Consistent with the imaging results, the delivery system with targeting moieties showed specific cytotoxicity (therapeutic effect) toward MDA-MB-468 cells. In control experiments with MCF-7 and L929 cells, to which the conjugate was not targeted, there were only rather weak fluorescence signals in the cells and essentially no cytotoxicity effects.

Wang et al. prepared hollow carbon dots through solvothermal treatment of BSA protein, where the pore size was estimated to be around 2 nm. The drug DOX was loaded onto the dots for both fluorescence imaging and delivery purposes. In A549 cells, the imaging results suggested the co-localization of carbon dots and DOX, with the dots mainly in the cytoplasm and DOX in the cell nuclei.

Mitra et al. grafted carbon dots to ZnO nanorod (ZCNP) for antibacterial and bioimaging applications. The resulting nanohybrid was effectively introduced into S. aureus cell in simple incubation, as observed in the fluorescence imaging under a microscope. The antibacterial efficacy of the nanohybrid was evaluated on S. aureus (Gram-positive) and E. coli (Gram-positive), with the results suggesting high antibacterial activities for both and in a concentration dependent fashion.

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These red/near-IR fluorescent dots were tracked \textit{ex vivo} and \textit{in vivo} for their biodistribution, excretion, and passive tumor uptake by using both near-IR fluorescence and positron emission tomography imaging techniques. The carbon dots were efficiently and rapidly excreted from the body after injection in different routes. Their blood clearance was quick, only one hour post intravenous injection. The retention times were somewhat longer after subcutaneous and intramuscular injections. The carbon dots were slightly trapped in liver, spleen, and lungs at one hour post intravenous injection. Very bright fluorescence was observed in kidneys. No meaningful fluorescence was detected in aforementioned samples after 24 h. The urine accumulation followed the sequence of intravenous > intramuscular > subcutaneous injection routes. For the three injection models, tumor uptakes of the carbon dots were also found (Fig. 9). In a related study, Huang et al. attached the fluorescence dye Ce6 to carbon dots to impart red fluorescence emissions through FRET. Indeed, the conjugate of Ce6–carbon dots could be excited at 430 nm for red fluorescence emissions (668 nm). For imaging and photodynamic therapy, the conjugate could be swallowed by cells, and under laser irradiation the cell death was observed. After intravenous injection, the accumulation of the conjugate in tumor was detected. As a result probably, the laser treatment of the mice exposed to the conjugate of Ce6–carbon dots significantly inhibited the tumor growth.

4. Graphene quantum dots for bioimaging

More recently, fluorescent GQDs have attracted great interest. As carbon-based nanomaterials, GQDs share some spectroscopic and other characteristics with carbon dots. In fact, Cao et al. pointed out that GQDs commonly referred to in the literature could be understood in two separate yet related categories, with their corresponding fluorescence emissions due to bandgap transitions in isolated $\pi$-domains (or often called “sp$^2$ islands”) or structural defects. However, the creation of sp$^2$ islands is typically facilitated or accompanied by defects, especially for the fact that the defect-derived fluorescence is strong, so that the observed fluorescence emissions in GQDs are either dominated by or substantially contaminated with defect-related contributions. It led the conclusion that “it is all about defects” (Fig. 10), with the notion that a GQD may be considered to a significant extent as a two-dimensional collection of multiple carbon dots.

GQDs were mostly prepared from graphene oxides (GOs), for which hydrothermal processing was widely adopted. In some cases, the as-processed sheets were reduced to further enhance the fluorescence, and more significant enhancements were achieved through the passivation of GQDs by polymers and other species. The functionalization
chemistry was also used to alter the fluorescence colors of GQDs.\textsuperscript{128,129} In the work by Zhu et al., for example, GQDs were grafted with alkylamines, in comparison with those reduced by NaBH\textsubscript{4}. Fluorescence colors in the former were different from those in the latter, despite their similar sizes (Fig. 11).\textsuperscript{128}

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Fig. 10  Top: (right) isolated sp\textsuperscript{2} islands in a graphene sheet and a photo showing the associated bandgap fluorescence in solution, and (left) a multiple-layer graphene piece with defects. Bottom: (left) a carbon nanoparticle with surface defects, and (right) fluorescence emission color variations in carbon dots (from ref. 21).

Fig. 11  (a) Preparation routes of GQDs with different functionalizations. (b) AFM images (left) and FL spectra (right) of GQDs (from ref. 128).
Bottom-up methods were also developed for the preparation of GQDs.\textsuperscript{130–132} For example, Liu \textit{et al.} used hexa-peri-hexabenzocoronene (HBC) to synthesize GQDs,\textsuperscript{130} where HBC pieces were stacked and converted into graphite, followed by oxidation and functionalization. The resulting GQDs exhibited similar fluorescence characteristics to those found in carbon dots. Shen \textit{et al.} functionalized GO-derived GQDs with PEG\textsubscript{1500N}, then the reduction with N\textsubscript{2}H\textsubscript{4}, and found significant upconversion fluorescence.\textsuperscript{18}

GQDs were applied to fluorescence imaging of cells and tissues in a conceptually similar fashion to that with carbon dots highlighted above. For example, Zhu \textit{et al.} reported that MG-63 cells could be labeled by GQDs, with green or yellow fluorescence emissions found in the cytoplasm at 405 nm or 488 nm excitation, respectively.\textsuperscript{133} The same group also used functionalized GQDs for cell imaging under upconversion fluorescence conditions.\textsuperscript{128} The results again suggested that the dots were localized in the cytoplasm of MC3T3 cells.

Peng \textit{et al.} prepared GQDs from carbon fibers for imaging human breast cancer cell T47D.\textsuperscript{134} Upon the incubation for 4 h, agglomerated green GQDs were found to be surrounding each cell nucleus. Sun \textit{et al.} used chemically and photochemically reduced GQDs to label A549 cells.\textsuperscript{135} Blue fluorescence was observed around the cell nucleus at 380 nm excitation. Zhang \textit{et al.} also reported that GQDs were localized in the cell cytoplasm in the fluorescence imaging of HeLa cells.\textsuperscript{136}

Hu \textit{et al.} prepared N-doped GQDs by hydrothermal treatment of GOs in the presence of ammonia for the imaging of HeLa cells. The N-GQDs were again trapped in the cell cytoplasm, with bright green fluorescence emissions observed at 405 nm excitation.\textsuperscript{137} Pan \textit{et al.} cut graphene into GQDs for cell imaging, and found that the GQDs were trapped in the nuclei of HeLa cells after 2 h incubation.\textsuperscript{138}

Xie \textit{et al.} used GQDs from the electrolysis of graphite in basic aqueous solution and then hydrazine hydrate reduction for imaging MCF-7 and A549 cells.\textsuperscript{139} Based on the observed yellow fluorescence emissions, GQDs readily entered the cells and localized in the cell cytoplasm, without significant attachment on the cell membrane. The same group also used the GQDs for the imaging of stem cells, including neurospheres cells (NSCs), and progenitor cells of pancreas and cardiac. The NSCs were visible as spheres consisting of a large nucleus surrounded by a small volume cytoplasm. No fluorescence was found in the cell nucleus. For progenitor cells of pancreas and cardiac, the fluorescence of GQDs was also found only in the cytoplasm.\textsuperscript{140}

Nurunnabi \textit{et al.} prepared fluorescent nanoparticles similar to GQDs for imaging \textit{in vivo}. The nanoparticles were introduced into nude mice through intravenous injection, with fluorescence emissions observed in the entire body 30 min post-injection. The fluorescence intensities remained for about 8 h, and decreased slowly to become very weak after 16 h. The results of \textit{ex vivo} imaging clearly showed the accumulation of the nanoparticles in liver, kidneys and spleen at 4 h post-exposure. Weak signals were also detected in heart and lungs. At 16 h post-exposure, the accumulation level decreased dramatically (Fig. 12).\textsuperscript{141}

Markovic \textit{et al.} evaluated the potential of GQDs for photodynamic therapy.\textsuperscript{142} According to the fluorescence imaging results, the GQDs were localized in the cytoplasm of U251 cells, consistent with the results of TEM and FACS. The photoexcitation of the GQDs induced autophagy, suggesting phototoxicity for potential uses in photodynamic therapy.\textsuperscript{142}

![Fig. 12](image-url) (a) Side and (b) front views of mice after intravenous injection of GQDs, and (c) \textit{ex vivo} images of dissected organs (reproduced from ref. 141).
5. Summary and perspective

Just based on the number of recent publications alone, carbon-based QDs have clearly become a new class of optical nanomaterials that compete with conventional semiconductor QDs for popularity, promising potentially competitive, performance-wise, yet nontoxic fluorescence bioimaging agents. There has also been growing evidence suggesting the critical role of defects in carbon dots and GQDs dictating their observed fluorescence properties and photoinduced processes in general, despite the fact that GQDs were originally pursued as a collection of isolated π domains on a single-layer graphene sheet. Consequently, carbon dots in which defects in the carbon nanostructure are obviously most abundant may serve to represent the majority of the GQDs and other fluorescent carbon nanomaterials of QD-like characteristics phenomenologically and mechanistically.

For fluorescence imaging of cells and tissues, carbon dots are advantageous in terms of their ready aqueous solubility, physicochemical and photochemical stabilities, high optical performance and non-blinking, and more importantly excellent biocompatibility. A further proliferation of investigations on potential bio-applications of carbon dots in imaging and beyond may be expected. For carbon dots of enhanced performance, new and possibly unconventional approaches may be explored for the synthesis of carbon dots with bright fluorescence emissions emphasized in the red/near-IR spectral regions, thus more effective in tissue penetration and reducing the interference from background fluorescence. This may represent a major challenge for the future scope of research in both the further development (including mechanistic elucidation) and application of carbon dots as a new class of fluorescence bioimaging agents. Also in need are brightly fluorescent carbon dots of various sizes, shapes, and surface functionalities, including specific targeting moieties, for live cell imaging at high resolution. More studies on the use of carbon-based QDs as a platform for dual- or multi-modality bioimaging agents are desired and expected.

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