Peptide-Decorated Tunable-Fluorescence Graphene Quantum Dots

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ABSTRACT: We report here the synthesis of graphene quantum dots with tunable size, surface chemistry, and fluorescence properties. In the size regime 15–35 nm, these quantum dots maintain strong visible light fluorescence (mean quantum yield of 0.64) and a high two-photon absorption (TPA) cross section (6500 Göppert–Mayer units). Furthermore, through noncovalent tailoring of the chemistry of these quantum dots, we obtain water-stable quantum dots. For example, quantum dots with lysine groups bind strongly to DNA in solution and inhibit polymerase-based DNA strand synthesis. Finally, by virtue of their mesoscopic size, the quantum dots exhibit good cell permeability into living epithelial cells, but they do not enter the cell nucleus.

KEYWORDS: graphene quantum dots, tunable fluorescence, peptides, DNA binding, biological imaging, two-photon fluorescence

INTRODUCTION

Growing efforts have been focused on the use of graphene for biomedical applications such as drug/gene delivery,1,2 imaging,3–5 biological sensing,6–7 and as a biocompatible scaffold for cell culture and tissue engineering.8–9 Reducing the size of a graphene sheet to the nanoscale results in a graphene quantum dot (GQD), which has sparked excitement due to several significant merits desirable for bioimaging/biotargeting such as its optical properties, small size that is commensurate with biomolecules, and ease of functionalization. GQDs exhibit superior brightness10 and excellent photostability against photobleaching and blinking as compared to organic dyes and semiconductor quantum dots (QDs).11,12 Also, their chemical composition is milder than those of the traditional semiconductor QDs, which exhibit heavy metal ion toxicity issues. However, despite their mild chemical composition, toxicity is GQD-size dependent. Earlier studies have shown that ultrasmall particles (<6 nm) are able to enter mitochondria and produce physical damage, which leads to cell apoptosis, contributing to oxidative stress.13,14 Shang et al. reported that sub-10-nm nanoparticles are more toxic to live cells as compared to bigger size particles.15 It is well-known that particles smaller than 10 nm can freely diffuse to the nucleus16,17 as also reported with GQDs.18,19 Although different sized GQDs (5–16 nm) were reported, size purification of the polydisperse product using gel electrophoresis was required, which complicates the method.20 With this in mind, we set out to develop a simple method for larger GQDs and investigate their chemical functionalization, biological function, and applications in bioimaging.

We synthesize GQDs by exfoliating and chemically cutting a suspension of natural graphite, which is initially in the form of powder (<20 μm). Upon purification of the GQDs, the size distribution of which can be controlled by the cutting conditions, we observe that graphene-binding peptides21 do not form ordered monolayers, presumably due to the presence of oxygen surface defects. To study this, we fabricated graphene nanosheets on a silicon substrate using a Scotch tape exfoliation method and oxidized the nanosheets by immersing the substrate in hot nitric acid, a treatment similar to that of the substrate during GQD synthesis, and then reduced them using the reducing agent hydrazine. Upon hydrazine treatment, we found that the graphene’s surface morphology is effectively restored to pristine form, allowing the organization of self-assembling peptides into β-tape monolayers. Afterward, we treated GQDs with hydrazine, which facilitates peptide binding on GQDs. The resultant peptide-decorated GQDs (pGQDs) are water-soluble, stable, highly fluorescent, and size-tunable. Although peptide-conjugated semiconductor QDs have been reported for staining live human breast cells22 and for targeting individual proteins in living cells,23 and in vivo imaging of tumor vasculature,24 double coating of the QDs is required due...
to high toxicity. On the other hand, GQDs may offer reduced toxicity, and to our knowledge, peptide conjugation to GQDs has yet to be reported. Moreover, integrating GQDs and self-assembling peptides could play an important role due to possibilities to introduce biocompatibility and various biochemical/biological functions that may affect regenerative medicine and biotechnology.25−27

In previous papers, studies of interactions between larger, >200 nm, graphene oxide (GO) or reduced graphene oxide (rGO) and oligonucleotides revealed a stronger affinity of rGO to oligonucleotide than to GO.5,7,28 Herein, we probe the binding characteristics of both GQDs and pGQDs to DNA. We observe that uncoated GQDs do not have a particular affinity to DNA, whereas cationic peptide-decorated GQDs (pGQDs) ubiquitously bind to DNA molecules, as observed by atomic force microscopy (AFM), transmission electron microscopy (TEM), and a polymerase-based strand synthesis assay. Moreover, to demonstrate utility for bioimaging applications, we show that pGQDs readily penetrate living MCF-10A epithelial cells, whereas they do not enter the nucleus and lose their viability. Finally, two-photon absorption measurements reveal a high TPA cross section, useful for deep-tissue imaging at reduced phototoxicity levels as compared with single-photon excitation.

■ RESULTS AND DISCUSSION

Preparation of Multicolor Emitting GQDs and Chemical Analysis. To synthesize GQDs we used a two-step liquid-phase chemical exfoliation method, a scaleup method to give large quantities of exfoliated material.29,30 The process to obtain multiple-color GQDs from natural graphite powder is shown in Figure 1a. The dark brown dispersion of tattered graphite obtained in step 1 of the process turns to a golden color after step 2 and a dialysis-based purification step. A photograph of multiple-color fluorescence from different average diameter GQDs under UV lamp irradiation is shown in Figure 1b. The average particle diameter of a series of the multiple-color GQDs as a function of oleylamine (OA) concentration is presented in Figure 1c, whereas in the Supporting Information we show AFM and TEM images and fluorescence spectra for a series of the GQDs (see Figures S1−S4). Control over the average GQD size is obtained by adjustment of the OA fraction in the preparation step, as detailed in Table 1 (see more details in the Experimental Section).

![Figure 1. Synthesis process and characterization of GQDs: (a) two-step preparation process of multiple color GQDs under UV excitation; (b) image of the fluorescence from different vials that contain different GQD sizes, illuminated with a UV lamp; (c) particle size (diameter) as a function of OA concentration (inset) (dashed square) is a high-resolution TEM image, showing a periodic structure that consists of peptide rows with spacing (3 ± 0.5) nm); (d) size (diameter) distribution of green emission GQDs before and after decoration with peptide from TEM ((inset) TEM image of GQDs (scale bar = 200 nm)); (e) emission spectra showing slight red shift in GQD peak by 8 nm after functionalization with peptide ((inset) fluorescence image of peptide-decorated green emission GQDs in water).](9379_F1){/}

### Table 1. Control Parameters of Synthesis Process with Average Diameter of a Series of Graphene Quantum Dots Shown in Figure 1b

<table>
<thead>
<tr>
<th>nanocrystal</th>
<th>average diameter (nm)</th>
<th>oleylamine (vol %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GQD1</td>
<td>14 ± 4.1</td>
<td>40</td>
</tr>
<tr>
<td>GQD2</td>
<td>19 ± 2.9</td>
<td>30</td>
</tr>
<tr>
<td>GQD3</td>
<td>26 ± 3.6</td>
<td>20</td>
</tr>
<tr>
<td>GQD4</td>
<td>30 ± 6.7</td>
<td>10</td>
</tr>
<tr>
<td>GQD5</td>
<td>34 ± 6.9</td>
<td>5</td>
</tr>
</tbody>
</table>

*Fraction of oleylamine in the octadecene solvent is reported.*

Experimental Section). Prior to any further manipulation and/or analysis, the as-synthesized GQDs were treated using a 50−60% hot hydrazine hydrate solution for chemical reduction/defect repair (more details can be found in the Experimental Section).

Interestingly, we obtained very high emission quantum yields (QY) with a mean of 0.64 for the GQD samples (see Figure S5 for details). To explore the reason behind these high observed QY values, we turn to their chemical composition, in particular, to their nitrogen content, which can strongly affect the GQD’s optical properties. In Figure 2a we present X-ray photoelectron spectroscopy (XPS) plots of four of the samples we prepared, labeled GQD1 through GQD4 in order of increasing diameters (see Table 1 for details). Samples were prepared by evaporating...
forms of doped nitrogen in the graphene quantum dots may account for the increased quantum yield and spectral shifts of the quantum dots.

Peptide Functionalization of GQDs. To prepare watersoluble, biocompatible, and cationic- GQDs, we utilized two different graphite-binding peptides eight amino acids in length that alternate four hydrophobic residues valine (V) or phenylalanine (F) with positively charged lysine (K), that is, KVKVKVKV and KFKFKFKF. On the basis of our prior results, these peptides self-assemble on a graphitic surface into extremely stable, flat, and ordered β-tape crystalline domains, in which cationic hydrophobic residues face outward toward the aqueous phase to allow DNA binding. The resulting cationic pGQDs are stable (see Figure S9) and highly soluble in water and retain the characteristic optical properties of the original uncoated GQDs. The zeta-potential measurement of GQDs and pGQDs in water provided supporting evidence for the surface functionalization and stability of the particles (see Figure S10). The large change in zeta potential from −6.79 to 42.9 mV is related to the presence of cationic peptide on the GQD surface. The inset in Figure 1c (dashed square) is a high-resolution TEM image (1% uranyl acetate-stained), which reveals a periodic structure that consists of peptide rows with a spacing of 3 ± 0.5 nm, which matches computational predictions. Figure 1d compares TEM-based diameter distributions (histograms) of GQDs before and after peptide functionalization, whereas the inset shows a representative TEM micrograph of the GQDs. We find that the peptide coating increased the mean GQD diameters by ~4 nm, which was also corroborated by AFM measurements (see Figure S11e). Finally, Figure 1e compares the emission spectra of GQDs and pGQDs. A slight (8 nm) red shift in GQD emission (from 482 to 490 nm) was observed following surface functionalization, whereas the inset shows a representative fluorescence intensity was retained upon GQD surface modification, as shown in the image inset in Figure 1e for pGQDs at similar concentration as the GQDs in Figure 1b.

High-resolution AFM and TEM images of the GQDs and pGQDs dispersed in water suggest that GQDs are well-dispersed, and diameter distributions histogram plots confirm that the peptide coating is uniform and does not lead to significant GQD aggregation (see Figure S11). Nanoscope software-based analysis of AFM images of representative GQDs (N = 1150) yields the topographic height distribution, and the mean height is found to be 1.6 ± 0.14 nm (see Figure S11h). This is further confirmed by Raman spectroscopy: Figure S11g shows a representative Raman spectrum of the GQDs, which exhibits two prominent Raman features, A D band (1354 cm−1) and a G band (1584 cm−1), which are generally assigned to E2g phonon involving the sp2 bonds between carbon atoms, respectively. The former Raman band is quite sharp within disordered graphite. Hence, a smaller I D /I G peak intensity ratio (0.74) in the Raman spectrum can indicate lower defects and disorders of the structures. Interestingly, unlike the GQDs synthesized with previously reported methods, the 2D band of as-generated GQDs is relatively strong, similar to that of high-quality larger area graphene. Moon et al. also reported the presence of a 2D band in GQDs synthesized using the CVD method. The ratio...
of the intensity of the G band to that of the 2D band is 1.23 for three-to-four-layered GQDs. These features indicate that the as-generated GQDs have not only high quality but also high surface area, which facilitates ligand attachment and loading of antibodies to the surface for biotargeting and biosensing applications.1,11

Recuperation of Nanographene Surface Enables Peptide Organization. In Figure 3 we present AFM images and Raman spectra that illustrate the impact of hydrazine treatment39,40 on the ability of peptides to organize on the graphene surface. Briefly, graphene flakes fabricated on a silicon substrate using Scotch tape exfoliation from graphite were oxidized by immersing the substrate in hot nitric acid (65%) at 100 °C for 1 h, a treatment similar to that used for the flakes during GQD synthesis. Subsequent peptide deposition yielded some peptide adsorption, yet no organization was observed after prolonged immersion, as shown in Figure 3c. The inset shows a spatial Fourier transform of the image, which further confirms that no organization is detected in the image. In contrast, when the oxidized flakes were hydrazine-treated prior to peptide deposition, we observe peptide organization to form β-tapes, as shown in Figure 3d. Even on a flake as small as 10 nm, peptide organization can be seen in the AFM image (see blue circle). Peptide organization is further confirmed by the inset FFT pattern, which reveals the characteristic C3-symmetry hexagonal pattern, with 4.2 ± 0.2 nm spacing in water that corresponds to the β-tape spacing.21 Peptide organization also occurred on single flakes, such as the 0.8 nm thick flake highlighted in the image (one-layer graphene and peptide monolayer, see red arrow). Raman spectra of the oxidized and hydrazine-treated graphene before and after exposure to peptide are shown in Figure 3, panels e and f, respectively. For this measurement, we obtained Raman spectra from the same flake after each treatment/coating process (confirming with optical microscopy). As shown in Figure 3f, the 2D band of hydrazine-treated graphene is blue-shifted by 12 cm−1 upon incubation with the peptide, whereas no shift is detected when the peptide is exposed to oxidized graphene (Figure 3e). This shift in the 2D band can be attributed as a slight doping effect with the adsorption of peptide (adsorbate) on graphene.41,42 Peptide adsorption produces increased signal at both the G and 2D bands. The peak near 1351 cm−1 represents the D band with an ID/IG ratio that is proportional to defect density.55 The spectra show that incubation with the peptide does not damage...
the graphene lattice, consistent with the expected noncovalent interaction between graphene and peptide. For comparison, Raman spectra of pristine, oxidized, and chemically reduced single-layer graphene are shown in Figure S12.

**DNA Binding Properties of Peptide-Decorated GQDs.** To use GQDs for biomedical applications, it is critically important to understand their interaction with cells, tissues, and biomolecules. We designed the highly cationic nature of the pGQDs to exhibit a strong affinity toward anionic DNA molecules. To study this, we probed the binding characteristics of both GQDs (diameter = 19 ± 2.9 nm) and pGQDs (diameter = 23 ± 3.0 nm) with DNA. Panels a and b of Figure 4 show AFM topographic images on mica of 2000 bp linear double-stranded DNA, as well as DNA that was exposed to GQDs, respectively (TEM images of equivalent samples are shown in Figure S13, panels a and b, respectively). We observe that unmodified GQDs do not specifically bind to the DNA molecules, as evidenced by random localization of GQDs with respect to the DNA backbones in the obtained images (see Figure 4b and Figure S13b).

It is expected that self-assembling amphiphilic peptides will form a monolayer structure by specifically organizing on the GQD surface to expose DNA-binding moieties. To quantify the interaction/binding specificities between pGQDs and DNA, we compare two samples, one in which pGQDs and DNA were incubated overnight prior to imaging and another in which immobilized DNA molecules on mica surface were exposed to pGQDs for 30 min (Figure 4c,d). In both experiments, structural changes of dsDNA upon incubation with pGQDs are observed. Importantly, we do not find any evidence of breaking or damaging of DNA molecules by both GQDs and pGQDs, which is different from earlier results with semiconductor QDs and nanoparticles because of leaching of heavy metal ions. An earlier study on GQDs revealed that intravenously injected larger GQDs (40 nm) alleviate immune-mediated liver damage. High-quality micrographs revealed that pGQDs bind internally and at DNA termini, forming bridges and inducing bends and interlock structures frequently involving DNA loops. We frequently observed the formation of such DNA loops under high pGQDs concentrations and/or long incubation times. A representative image of network formation with several loops is shown in Figure S14b, where arrows indicate loops and bridges promoted by cooperative and concurrent binding of pGQDs. Similar structural changes to DNA upon pGQD binding were detected using TEM (see Figure S15). Dynamic light scattering (DLS) also provided supporting evidence for peptide functionalization and DNA binding (see Figure S16). The average diameters of the representative pGQDs with standard deviation from TEM, AFM, and DLS are 23 ± 3, 26 ± 4.6, and 35 ± 1 nm, respectively. For the DNA–pGQDs complexes, the average hydrodynamic diameter based on DLS is 46 ± 2 nm, which represents a significant increase.

UV–vis absorption and fluorescence spectra of the pGQDs and DNA-bound pGQDs are shown in Figure S17. A slight blue shift (8 nm) and an increase in emission intensity, along with an increase in full width at half-maximum (fwhm), were detected upon incubation of pGQDs with DNA. However, no change in the spectrum was detected upon incubation of GQDs with DNA, suggesting little to no interactions. The origin of fluorescence of GQDs has been attributed to several possibilities in the literature: surface groups, surface passivation, defects, change in surface chemistry, and fluorophores with different degrees of π-conjugation. In our case, a change in the pGQD spectrum upon incubation with DNA could result from charge transfer between cationic pGQDs and DNA. Lee et al. and Zhang et al. also reported the increase in fluorescence intensity of fluorochrome-functionalized Faraheme nanoparticle (FHNP) and g-C3N4 QDs with the interaction of DNA, respectively. The broadening of the fwhm could be due to a change in the electrostatic environment of the pGQDs upon binding to DNA.

**Persistence Length of DNA and DNA–pGQD Complex.** To further explore the interactions of DNA and pGQDs, we examined many single molecules of two samples, one in which pGQDs and DNA incubated overnight in solution and another incubated on a mica surface for 30 min (see Figure S14b,c). Interestingly, pGQD binding to DNA induces a bend to the DNA backbone, and the number of contact points increases when the DNA and pGQDs are both available to interact in solution. Intramolecular bridging causes formation of loops (green arrows, Figure S14c), whereas the intermolecular bridging mechanism apparently causes DNA molecules to cross-link (blue arrows, Figure S14c), and the multimolecular bridging mechanism induces several loops/rings and forms more complicated levels of organization. The type of bend observed upon pGQD binding is reminiscent of changes in DNA conformation induced by repressor proteins, enzymes, and restriction endonucleases. To perform a quantitative characterization of the persistence length of the DNA in the absence and presence of pGQDs, we carried out an automated DNA tracing script from AFM images and then further quantification and analysis using NCTRacer, a software
developed by the Neurogeometry laboratory at Northeastern University. To quantify AFM images, N = 140 DNA molecules in the absence and presence of pGQDs were traced and analyzed. The inset in Figure S14e presents a representative traced image of DNA. By fitting to the 2D WLC model (see eq 1 in the Supporting Information), a persistence length of 55 ± 3 nm for DNA (Figure S14e, green line) was calculated. This result is in agreement with the earlier reported persistence length of DNA calculated on the basis of AFM image in air. The correlation plot shows a decreased persistence length of DNA upon pGQD binding. The calculated value of the DNA–pGQD complex that was incubated overnight in liquid (Figure S14f, purple line, 29 ± 6 nm) is found to be slightly less than that when incubated on the surface (Figure S14e, red line, 35 ± 5 nm) using a 2D WLC model. As a control 2000 bp dsDNA (4.2 nM) was used. A decreased persistence length upon pGQD binding indicates an increase in the local flexibility of DNA. The uranyl acetate stained DNA–pGQD complex visualized by high-magnification TEM is represented in Figure S14d. In the micrograph, a string-like structure DNA (red arrow) providing at least 13 binding sites to pGQDs (blue arrows) is clearly visible.

Inhibition of Polymerase-Based DNA Strand Synthesis. The extensive organization of DNA and the pGQDs may interfere with, or prevent, the DNA from extending during the replication process, which in turn would affect gene expression. To clarify this phenomenon, we performed an additional experiment based on a phi29 DNA polymerase extension assay and examined the effect of both GQDs and pGQDs on DNA replication, as in the scheme of Figure 5a.

After a polymerase extension assay on a fluorescent primer, we purified the DNA and ran the products on a 18% polyacrylamide gel electrophoresis (PAGE). The scheme in Figure 5a shows a 111-mer stained DNA ladder (1), extension in the absence of GQDs (2), presence of GQDs (3, minute inhibition of replication), presence of pGQDs (4, no replication observed), and primer-hybridized 111-mer (lane 5). As shown in Figure 5b, inhibition in the extension of the primer indicates protection of the DNA by pGQDs.

Bioimaging with pGQDs. We first accessed the biological stability of the pGQDs in PBS and cell culture media to examine the surface prosperity and bioimaging capability of pGQDs under these conditions. In Figure 6a,b, we show confocal fluorescence images of pGQDs (diameter = 29 ± 4.3 nm) on a glass substrate from their dispersion either in PBS (a) or culture media (b) (scale bars = 20 μm); (c, d) bright field and fluorescence (e) and fluorescence (f) images of 3 μM pGQDs incubated with MCM-10A epithelial cells (e, f) bright field and fluorescence (e) and fluorescence (f) images of 4.5 μM pGQDs incubated with MCM-10A epithelial cells (pGQDs locate inside the cytoplasm and surround the nucleus rather than bind on the surface of the cells (scale bars (c–f) = 10 μm)). Laser excitation wavelength = 405 nm.

Figure 5. Phi29 DNA polymerase extension assay to assess the protection of DNA by pGQDs: (a) scheme showing 111-mer (blue) strand hybridized to a FITC-labeled 20-mer primer (orange), where the primer is extended using phi29 polymerase; (b) gel images show 50 bp DNA ladder (1), extension in the absence of GQDs (2, product is shown as dark band), presence of GQDs (3, minute inhibition of replication), presence of pGQDs (4, no replication observed), and primer-hybridized 111-mer (5).

Figure 6. Fluorescence images of pGQDs on glass substrate and in human mammary epithelial cells: (a, b) fluorescence confocal images of pGQDs dispersed on a glass substrate in either PBS (a) or culture media (b) (scale bars = 20 μm); (c, d) bright field and fluorescence (e) and fluorescence (f) images of 4.5 μM pGQDs incubated with MCM-10A epithelial cells (e, f) bright field and fluorescence (e) and fluorescence (f) images of 4.5 μM pGQDs incubated with MCM-10A epithelial cells (pGQDs locate inside the cytoplasm and surround the nucleus rather than bind on the surface of the cells (scale bars (c–f) = 10 μm)). Laser excitation wavelength = 405 nm.
with 1× DPBS. Bright field and fluorescence (merged) and fluorescence images of living cells shown in Figure 6c–f demonstrate that pGQDs permeate into cells and label within the cytoplasm but cannot penetrate nuclei because of their larger size, which may mitigate the GQD toxicity, although a more systematic study is outside the scope of this work.

**GQDs Exhibit Strong Two-Photon Fluorescence.** Two-photon absorption, a nonlinear optical process that is activated by near-infrared (NIR) femtosecond laser excitation, is expected to not only achieve a higher spatial resolution but also enable deep-tissue imaging due to tissue transparency in the NIR region. Therefore, we investigated the two-photon fluorescence characteristics of GQDs and pGQDs on a homemade optical setup using a Ti:sapphire laser with ∼100 fs pulse width as an excitation source. To efficiently excite GQD dispersed in water, the output wavelength was centered at 800 nm. Panels a and b of Figure 7 show the two-photon excited fluorescence (TPEF) spectrum of GQDs (3 μM or 0.22 mg/mL) and pGQDs (3 μM) dispersed in water under laser excitation (25 mW power). The inset in Figure 7a is a fluorescence image of solid GQDs dispersed on glass substrate, showing that different brightness of fluorescence originates from separate particles. To further investigate whether the observed green fluorescence of GQD and pGQDs originates from a TPA process with laser excitation in the NIR, the change in fluorescence intensity as a function of laser power was studied. As presented in Figure 7c, a quadratic relationship of fluorescence intensity with the excited laser power suggests the two-photon excitation is responsible for the green fluorescence. Figure 7d compares the excitation profile of two photons over a single photon. The emission spot images shown in Figure 7d were obtained in a dark environment by respectively passing continuous (405 nm) and focusing pulsed (800 nm) laser beams through the GQD sample in a cuvette. In the two-photon case, the cross section is clearly localized in a spot, whereas single-photon excitation excites everywhere in the sample where the laser beam hits. To assess the possibility of using GQDs for TPA-based applications, the TPA cross section of GQD dispersed in water was measured using riboflavin as a reference (see the Supporting Information for measurement details), and a large value of 6500 GM with excitation at 800 nm was achieved, far surpassing the commonly used fluorescent dyes having a range of 1−300 GM and multiphoton absorption fluorophores having range of 11−302 GM, and is even comparable to the cytotoxic CdSe and CdTe QDs dispersed in toluene, which have values in the range of 780−10,000 GM. The large value of two-photon absorption may be attributed to efficient charge transfer between the large in-plane π-conjugated system of GQD and strong electron-donating amine and hydroxyl groups, as revealed by FTIR measurements (see Figure S6).

### Conclusions

In summary, we have demonstrated here peptide-decorated GQDs with tunable size, fluorescence, and two-photon cross sections. First, we find that oxygen defects on the acid-treated graphene severely inhibit peptide organization, and the defects are repaired by treatment with hydrazine. Thus, a promising, highly stable, water-soluble, strong TPA and biocompatible optical probe pGQDs were introduced for the first time. We also demonstrated the strong DNA-binding properties of cationic peptide-modified GQDs, evidenced by strong complexation of the pGQDs with DNA and inhibition of polymerase-based replication. Benefiting from the proper size distribution and surface modification that could reduce oxidative stress cellular damages, our pGQDs can easily be introduced into mammalian cells for living cellular imaging. We believe that further programmed decoration of the GQDs with peptides that target a specific receptor or set of receptors can afford a variety of new functional materials, including catalysts, biomarker detectors, and drug delivery vehicles.
**EXPERIMENTAL SECTION**

Synthesis of GQDs. GQDs were synthesized by liquid-phase chemical exfoliation of natural graphite. In short, 5 mg of graphite power (<20 μm, Aldrich) was mixed with 25 mL of concentrated nitric acid (Acros Organics) and heated to 110 °C (hot-plate temperature) with stirring for 15 h. The resultant solution was quenched with 40 mL of water, resulting in the homogeneous dark brown exfoliated graphite dispersion (see Figure 1). Two milliliters of tannin graphite dispersion was added to the mixture of different volumes of 1-octadecene (95%, Aldrich) and oleylamine (90%, Acros) and heated to 210 °C with stirring for 3 h to obtain different sizes of GQDs (see Table 1 and details in the Supporting Information).

Hydrazine Treatment of GQDs. Five milliliters of hydrazine hydrate was added to the above resulting mixture after cooling to room temperature and then further heated to 220 °C (hot-plate temperature) with stirring for 3 h. Ten milliliters of methanol was added to the product solution, and GQDs were precipitated and isolated by centrifugation (4000 rpm for 20 min) and redispersed in methanol (10 mL). This process was repeated at least three times to get rid of excess hydrazine, 1-octadecene, and oleylamine. The final product dissolved in methanol was further dialyzed in a dialysis bag (Spectra/Por Biotec Cellulose Ester, 100–500 Da) against 1 L of methanol for 15 h to remove tiny fragments and chemical residues.

Peptide Functionalization of GQDs. A 67.2 μM peptide solution was incubated with the GQDs dispersed in water of concentration 3.3 μM (estimated from spectrophotometry using ε = 1.2 × 10^9 cm^-1 M^-1 cm^-1) for 12 h and followed by dialysis against 2 L of DI water for 15 h to remove free unconjugated peptide. Thus, a peptide-functionalized GQD complex with pGQDs was obtained. AFM, TEM, and polycrystalline gel electrophoresis were carried out to confirm the successful bioconjugation and further supported by DLS, fluorescence, and UV–vis experiments.

AFM Imaging of DNA–pGQD Nanocomplexes and Persistence Length. DNA (2000 bp) stock solution (100 ng/μL, 83 nM) was thawed to room temperature and diluted to 5 ng/μL (4.2 nM) with DI water. Diluted DNA (10 μL) was mixed with diluted pGQDs (2 μL, 0.51 μM) and incubated overnight in a vial for sufficient binding. Then 4 μL of the mixture was deposited on a freshly cleaved mica surface, which had already been exposed to 3 mM MgCl_2 for 10 min at ambient temperature. After 20 min of exposure, the mica surface was thoroughly rinsed with Milli-Q pure water and then blown dry with ultrapure nitrogen gas. For the surface incubation, DNA (4.2 nM, 5 μL) was first immobilized on the mica surface, and 2 μL of dialyzed pGQDs (0.51 μM) was introduced; 30 min was allowed to elapse for pGQDs to interact with DNA. After 30 min of incubation, the mica surface was gently rinsed with Milli-Q pure water and then blown dry with ultrapure nitrogen gas. AFM images were collected at ambient temperature using fast scan dimension AFM (Bruker, USA) in tapping mode. Silicon cantilever was used (force constant = 18 N/m, α = 9385 kHz). Images were processed using Nanoscope software. To find the persistence length of the DNA, each DNA molecule (from AFM images) was traced, quantified, and analyzed using NCTrace software, developed by the Neurogeometry laboratory at Northeastern University.

Transmission Electron Microscopy. TEM images were obtained using a JEOL 2010-PEG transmission electron microscope operated at 200 kV (for GQDs) and a JEOL JEM-1010 transmission electron microscope operated at 80 kV (for DNA, peptide, pGQDs, and DNA–pGQDs) in bright field mode. TEM samples were prepared by casting 4 μL of sample solution on the grid (300 mesh Cu with a Formvar film, Ted Pella). Before biological samples were deposited, the grids were treated with poly-L-lysine for 15 min, washed with three drops of DI water, and dried completely. One percent uranyl acetate was used for staining the samples prior to TEM imaging.

Raman of GQDs. A highly concentrated colloidal solution of GQDs was obtained from as-synthesized GQDs by removing some portion of buffer using reduced-pressure evaporation in a vacuum. Then, 10 μL of the highly concentrated solution was cast on the Si/\SiO_2 substrate. The Raman spectrum was obtained by using a Ranishaw Ramascope with a 488 nm laser as excitation source.

UV–Visible, Fluorescence, and XPS Measurements. Absorbance spectra were acquired using a Varian Cary 100 scanning UV–vis spectrophotometer and using a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, USA), and fluorescence spectra were recorded using an F-2500 fluorescence spectrophotometer (Hitachi). Measurements of both absorption and fluorescence spectra were performed at room temperature, and quartz cuvettes were used for fluorescence measurement. XPS measurement was completed on the K-Alpha XPS system from Thermo Scientific at Harvard University’s Center for Nanoscale Systems. The probe for the measurement was the Al Kα X-ray line with energy at 1.4866 keV and an X-ray spot size of 400 μm. A flood gun, which supplies low-energy electrons and ions, was used throughout the experiment for sample surface charge compensation. Survey spectra and high-resolution scans were acquired for each GQD sample.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.6b16364.

AFM, TEM, FTIR, and fluorescence spectra of a series of the GQDs; diameter distribution from DLS, zeta potential, and stability of pGQDs; Raman spectra; AFM and TEM images of DNA–GQD and DNA–pGQD complexes; persistence length; optical absorbance spectra (PDF)

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The authors declare no competing financial interest.

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