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Graphene Quantum Dots for Bioanalytical Sensors and Biochemical Imaging Applications

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Graphene comprises a single atomic layer of carbon atoms; it is a semimetal with zero-energy gap. Graphene quantum dots (GQDs), carbon nanodots (CNDs) and polymer dots (PDs) are nanometer-sized variants of graphene. Their optoelectronic properties are capturing the imagination of many engineers and scientists. A monograph written by Güçlü, Potasz, Korkusinski and Hawrylak introduced the reader to this exciting and rapidly evolving field of GQDs and carbononics [1]. Fabrication of GQDs and their single-particle optical properties were described. By lateral size quantization, the gap in GQDs can be tuned from zero to UV. A review article authored by Bacon, Bradley and Nann gave an overview of the synthesis methods, energy-conversion properties and state-of-the art applications for this advanced material [2]. Owing to the exceptional physicochemical properties, high photostability and biocompatibility of GQDs, more exciting developments in this rapidly evolving field and biological applications were emphasized by Zheng, Ananthanarayanan, Luo and Chen [3]. They envisioned that CNDs might revolutionarily change the landscape of biomedical research. Early *in vitro* experiments showed that GQDs exhibit very low cytotoxicity owing to their ultra-small size and high oxygen content; *in vivo* biodistribution experiment of GQD revealed no material accumulation in main organs of mice and fast clearance of GQD through kidney [4]. However, excitation and emission at long wavelengths were particularly desired for deep tissue bioimaging.

The difficulty with production of high-quality GQDs with mono-disperse size and chemical functionality was resolved over the last several years. In a top-down procedure, coal or anthracite was dispersed in sulfuric acid and nitric acid [5]. The dispersion was sonicated and then heated at 100°C for 1 day. The clear brown-red solution was then cooled in an ice-water bath and diluted with deionized water. The solution was then dialyzed in bag against deionized water for 3 days to obtain GQDs. A facile bottom-up method for the synthesis of highly fluorescent GQDs was developed by Julia Zhao and co-workers using a heating mantle for the pyrolysis of L-glutamic acid [6]. The resultant GQDs showed strong blue, green and red luminescence under irradiation with ultra-violet, blue and green light, respectively. Moreover, the GQDs emitted near-infrared (NIR) fluorescence in the range 800-850 nm with a large Stokes shift of 455 nm, providing a significant advantage for the sensitive determination and imaging of biological targets. Wang et al. reported the gram-scale synthesis of single-crystalline GQDs by a facile molecular fusion route under mild hydrothermal conditions [7]. The synthesis involves the nitration of pyrene followed by hydrothermal treatment in alkaline aqueous solutions,

where alkaline species play a crucial role in tuning their size, functionalization and optical properties. Precise dimension control of GQDs produced by chemical synthesis methods was difficult to achieve and usually provided a range of sizes from 3 to 25 nm. Chua et al. used fullerene C60 as starting material, due to its well-defined dimension, to produce very small GQDs (2-3 nm) [8]. Treatment of fullerene C60 with a mixture of strong acid and chemical oxidant induced the oxidation, cage-opening, and fragmentation processes of fullerene C60. The quantum dots exhibited strong luminescence properties, with the highest intensity at 460 nm under a 340 nm excitation. Ye et al. engineered the bandgaps of photoluminescent GQDs synthesized from anthracite by controlling the size of GQDs [9]. A facile one-step chemical synthesis using successively higher temperatures to render smaller GQDs. Using these methods, GQDs were synthesized with tailored sizes and bandgaps. The GQDs emit light from blue-green (2.9 eV) to orange-red (2.05 eV), depending on size, functionalities and defects. These findings provide a deeper insight into the nature of coal-derived GQDs and demonstrate a scalable method for production of GQDs with the desired bandgaps.

While it can be expected that bioanalytical applications will rapidly evolve, basic research on the fundamental mechanism of GQDs luminescence must precede to gain a better understanding of their commercial potential. More work is needed toward the understanding of the electronic structure and optical properties of GQDs. Environmentally benign molecular devices will likely be advanced across a wide range of fields. A novel fluorescent sensor based on GQDs was synthesized by Zhou, Qua, Zeng, Zhou and Shi for the determination of para-nitrophenol (4-NP) in water samples [10]. A MIP layer was anchored on the silica-coated GQDs using 3-aminopropyltriethoxysilane as functional monomer and tetraethoxysilane as crosslinker. Due to resonance energy transfer, the fluorescence of the MIP-coated GQDs composite could be efficiently quenched when 4-NP molecules rebound to the imprinted sites. One study demonstrated that a novel Au NPs–N-GQDs nanocomposite was promising for fabrication of non-enzymatic hydrogen peroxide biosensors [11]. The formation of hybrid was achieved by just mixing the N-GQDs and $HAuCl_4 \bullet 4H_2O$ without addition of reductant or surfactant. The Au NPs–N-GQDs exhibited high sensitivity and selectivity for electrochemical detection of H_2O_2 with a low detection limit of 0.12 μM. A fluorescent sensor was developed for dopamine (DA) detection with high sensitivity and selectivity, based on polypyrrole/GQDs core/shell hybrids [12]. The fluorescence intensity decreased with the addition of DA, with a detection limit of 10 pM. Novel conjugates of GQDs and gold nanoparticles are synthesized by Ting, Ee, Ananthanarayanan and Chen for sensitive electrochemical detection of Hg^{2+} ions to achieve an ultralow detection limit of 0.02 nM [13]. A facile bottom-up method for the synthesis of highly fluorescent nitrogen-doped GQDs was developed by Lin et al. via a one-step pyrolysis of citric acid and tris(hydroxymethyl) aminomethane [14]. The obtained N-GQDs emitted strong blue fluorescence under 365 nm UV light excitation with a quantum yield of 59%. The fluorescence intensity of the N-GQDs could be greatly quenched by 2,4,6-trinitrophenol (TNP), with a detection limit of 0.3 μM, in natural water samples. A fluorescence resonance energy transfer biosensor based on GQDs was developed for *Staphylococcus aureus* gene sequence detection [15]. Capture probes were immobilized on GQDs and reporter probes were conjugated with Au nanoparticles. The target DNA oligos co-hybridized with capture probes and reporter probes to form a sandwich structure and trigger the FRET effect. A detection limit of 1 nM was achieved for *Staphylococcus aureus* gene detection. Bhatnagar, Kumar and Kaur developed a GQDs FRET based sensor for heart attack detection [16]. The immunosensor response time was 10 s and the total time for detection was only 10 min with a detection limit as low as 0.2 pg/mL.

Zhou et al. reported the development of a multifunctional nanocarrier consisting of paramagnetic GQDs for the delivery of doxorubicin using folate as a targeting ligand [17]. This nanocarrier exhibited negligible cytotoxicity and excellent biocompatibility. More importantly, strong therapeutic activity was achieved by loading doxorubicin onto the nanocarrier surfaces through π–π stacking and hydrophobic interactions. Approximately 80% of the loaded doxorubicin was released under mild acidic conditions (pH 5.0). Flow cytometry analysis and confocal laser scanning microscopic observation showed that these nanocarriers were efficiently taken up by the cancer cells overexpressing folate receptors. However, studied the effect of GQDs on the immune system and their results showed that GQDs induce ROS generation, apoptosis, autophagy, and inflammatory response via p38MAPK and NF-κB mediated signaling pathways in THP-1 activated macrophages [18]. Violi, Kotov and co-workers reported that covalent attachment of l/d-cysteine moieties to the edges of GQDs led to their helical buckling due to chiral interactions at the crowded edges [19]. Exposure of liver HepG2 cells to l/d-GQDs reveals their general biocompatibility and a noticeable difference in the toxicity of the stereoisomers.

Zhu et al. reviewed four mechanisms of photoluminescence for GQDs, CNDs, and PDs [20]. The quantum confinement effect, or conjugated π-domains, is determined by the carbon core; the surface state is determined by hybridization of the carbon backbone with chemical groups; the molecule state is determined solely by the fluorescent molecules connected on the surface or interior; and the crosslink-enhanced emission effect. Exciting progresses on CND- and GQD-based optoelectronic and energy devices, such as light emitting diodes, solar cells, photodetectors, photocatalysis, batteries and supercapacitors were reviewed by Li et al. [21]. McGuire, Hawrylak and co-workers presented transient absorption measurements and microscopic theory of biexciton binding in triangular colloidal GQDs consisting of 168 sp^2-hybridized C atoms [22]. They observed optical transitions from the lowest orbitally dark singlet exciton states to states below the energy of an unbound dark+bright singlet-exciton pair. Through microscopic calculations of the low-energy exciton and biexciton states via tight-binding, Hartree-Fock, and configuration interaction methods, the spectra revealed a biexciton consisting primarily of a dark-bright singlet-pair bound by ~0.14 eV. A novel metal-free N-GQDs/g-C_3N_4 catalyst was designed and synthesized by Luo, Suib and co-workers [23]. It showed much better photocatalytic activity for H_2 evolution

from water splitting than that of g-C_3N_4 due to the unique and multiple roles of N-GQDs. Valizadeh and co-workers modified quantum dots by converting the hydroxyl functional groups to nitrite groups using sodium nitrite [24]. The modified GQDs were used as a catalyst and reagent for diazotization reaction of aniline derivatives. Subsequent azo-coupling reactions with active aromatic compounds produced azo-dyes in excellent yields.

More recent works continue to advance the frontier of science in this blooming research field. New findings include light absorption in graphene causing a large change in electron temperature due to the low electronic heat capacity and weak electron–phonon coupling. El Fatimy et al. have shown that quantum dots of epitaxial graphene on SiC exhibited an extraordinarily high variation of resistance with temperature (higher than 430 MΩ K^{-1} below 6 K). Combining a high responsivity of 1×10^{10} V W^{-1} with an extremely low electrical noise-equivalent power ($\sim 2 \times 10^{-16}$ W $Hz^{-1/2}$ at 2.5 K), these quantum dot bolometers demonstrated good performance at temperatures as high as 77 K [25]. On a brave front, Gupta, Fakhri and co-workers synthesized GQDs using the microwave-assisted hydrothermal route for the removal of oxamyl (a noxious pesticide) from aqueous solutions [26]. The adsorption equilibrium and kinetic data were well fitted with the Langmuir isotherm and pseudo-second-order kinetic model. All these discoveries speak to the excitement of scientific adventures in the world of quantum dots, particularly for a better knowledge of graphene!

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