



Nanobiosensing with graphene and carbon quantum dots: Recent advances

Brandon K. Walther^{1,2,†}, Cerasela Zoica Dinu^{3,‡}, Dirk M. Guldi^{4,¶}, Vladimir G. Sergeyev^{5,||}, Stephen E. Creager^{6,|}, John P. Cooke^{1,2,§}, Anthony Guiseppi-Elie^{1,2,7,*}

¹ Biosensors and Biochips (C3B[®]), Department of Biomedical Engineering and Department of Electrical and Computer Engineering, Texas A&M University, College Station, TX 77843, USA

² Department of Cardiovascular Sciences, Houston Methodist Institute for Academic Medicine and Houston Methodist Research Institute, 6670 Bertner Ave., Houston, TX 77030, USA

³ Department of Chemical and Biomedical Engineering, West Virginia University, Morgantown, WV 26506, USA

⁴ Department of Chemistry and Pharmacy, Friedrich Alexander University Erlangen-Nürnberg 91058 Erlangen, Germany

⁵ Department of Chemistry, Lomonosov Moscow State University, Leninskie Gory 1-3, Moscow 119991, Russian Federation

⁶ Department of Chemistry and Department of Materials Science and Engineering, Clemson University, Clemson, SC 29634, USA

⁷ ABTECH Scientific, Inc., Biotechnology Research Park, 800 East Leigh Street, Richmond, VA 23219, USA

Graphene and carbon quantum dots (GQDs and CQDs) are relatively new nanomaterials that have demonstrated impact in multiple different fields thanks to their unique quantum properties and excellent biocompatibility. Biosensing, analyte detection and monitoring wherein a key feature is coupled molecular recognition and signal transduction, is one such field that is being greatly advanced by the use of GQDs and CQDs. In this review, recent progress on the development of biotransducers and biosensors enabled by the creative use of GQDs and CQDs is reviewed, with special emphasis on how these materials specifically interface with biomolecules to improve overall analyte detection. This review also introduces nano-enabled biotransducers and different biosensing configurations and strategies, as well as highlights key properties of GQDs and CQDs that are pertinent to functional biotransducer design. Following relevant introductory material, the literature is surveyed with emphasis on work performed over the last 5 years. General comments and suggestions to advance the direction and potential of the field are included throughout the review. The strategic purpose is to inspire and guide future investigations into biosensor design for quality and safety, as well as serve as a primer for developing GQD- and CQD-based biosensors.

* Corresponding author.

E-mail addresses: Walther, B.K. (brandonwalther1@tamu.edu), Dinu, C.Z. (Cerasela-Zoica.Dinu@mail.wvu.edu), Guldi, D.M. (dirk.guldi@fau.de), Sergeyev, V.G. (sergeyev@genebee.msu.ru), Creager, S.E. (screage@clemson.edu), Cooke, J.P. (jpcooke@houstonmethodist.org), Guiseppi-Elie, A. (guiseppi@tamu.edu).

[†] 0000-0002-4086-8897.

[‡] 0000-0002-6474-6771.

[¶] 0000-0002-3960-1765.

^{||} 0000-0002-8200-5993.

[|] 0000-0002-7412-2268.

[§] 0000-0003-0033-9138.

[®] 0000-0003-3218-9285.

Introduction

Graphene and carbon quantum dots (respectively, GQDs and CQDs) are recently discovered nanomaterials finding utility in the development of next-generation biosensors owing to their exceptional physical, chemical, and electronic transport properties [1,2]. CQDs were first isolated and characterized in 2004 by Xu et al. [3] and GQDs a few years later (2008–2010) [4–8]. In recent years, there has been an explosion of reports on biotransducer designs and biosensing applications using these

nanomaterials to facilitate, improve, and/or otherwise develop novel approaches for analyte detection and monitoring. This review focuses specifically on the use of GQDs and CQDs in the context of biotransducer development, with emphasis on how their unique physical and chemical properties enable previously unexplored avenues for biosensor innovation. There are already exceptional reviews covering material synthesis and general applications of these nanomaterials [1,2,9–11], however, no review critically analyzes the existing literature while specifically focusing on biotransducer design and interfacial engineering. Thus, the scope of this review will include an introduction that defines biosensors broadly and nano-enabled biotransducers specifically, fundamental quantum properties of GQDs and CQDs, and how they may be used to improve biosensing technologies – synthesis methods will be generally excluded in this specific discussion. The literature itself will be reviewed with emphasis on current areas of progress and specific needs or directions the scientific community may take when developing these nano-enabled biosensor systems (see Table 1).

Biosensing and nanotechnology

Biosensors are micro-analytical systems. Biosensing is a subfield of the general field of analytical sensor development and applications, with a specific focus on incorporating molecular biological recognition entities into the detection modality. The term “biosensor” refers to any sensing system that integrates a biological recognition component to confer analyte specificity at the molecular level. Biosensors themselves can be divided into three distinct components: the *biotransducer* which combines the biological recognition element that confers molecular specificity to the analyte of interest; the *physiochemical transducer* that is coupled with the molecular recognition entity; the *instrumentation* subsystem which interrogates the biotransducer to processes received signals; and the *readout device*, a subsystem which provides tangible output in the form of actionable information [12]. It is essential to emphasize that the biotransducer is one sub-system, representing the contraction of the terms “biological recognition” and “signal transduction” and is thus reflective of the functional intimacy of these two parts (Fig. 1) [13–15].

Nanobiosensing is an extension of the foregoing conceptual framework which integrates nanomaterials into the biosensing regimen in some capacity in order to alter the chemical binding profiles and material composition, enable a unique detection modality, improve sensitivity and/or specificity, or impart a fundamentally new property to the overall system [16,17]. This involves, in an overwhelming majority of cases, integration of nanomaterials into the biotransduction component [18–22].

General classes of biotransducers and sensing strategies

General classes of biotransducers

Biotransducers can be classified based on the way in which the biological recognition component is interfaced with the physiochemical transducer [14,23]. A generation I biotransducer is one in which the analyte detection is derived from the production or consumption of a signal-producing molecule whose chemical potential elicits a response proportional to the chemical potential of the analyte of interest. A generation II biotransducer is one in which a mediator molecule or immobilized membrane

TABLE 1

Commonly used acronyms.

Acronym	Meaning
<i>Physical/Chemical</i>	
QD	Quantum Dot
GQD/CQD	Graphene/Carbon Quantum Dot
GO	Graphene Oxide
PEC	Photoelectrochemical
ECL	Electrochemiluminescence
HOMO	Highest Occupied Molecular Orbital
LUMO	Lowest Unoccupied Molecular Orbital
FRET	Fluorescent (Förster) Resonance Energy Transfer
DET	Direct Electron Transfer
LOD	Limit of Detection
R _{CT}	Charge Transfer Resistance
CV	Cyclic Voltammogram
<i>Biological</i>	
DNA/RNA	(Deoxy)Ribonucleic Acid
ss/ds	Single Stranded/Double Stranded (DNA/RNA)
Ab	Antibody
Ig(A/D/E/G/M)	Immunoglobulin (Class) – Specific Ab classes
GOx	Glucose Oxidase
HRP	Horseradish Peroxidase
AChE	Acetylcholinesterase
ATP	Adenosine Triphosphate
RTqPCR	Reverse Transcription Quantitative Polymerase Chain Reaction
ELISA	Enzyme-Linked Immunosorbent Assay
CEA	Carcinoembryonic Antigen
EpCAM	Epithelial Cell Adhesion Molecule
IL-	(descriptor)
Interleukin	

is used to enhance the physiochemical transduction of the signal-producing molecule. Lastly, generation III biosensors eliminate the need for mediators by directly interfacing the action of the biomolecular recognition component with the physiochemical transducer, allowing for ideally a 1:1 readout of biological recognition reaction with the transducer’s signal production. Glucose biosensing is an excellent concrete example which highlights the various generational developments of biotransduction [24,25]. Then pioneering work of Leland Clark for glucose biosensing employed the enzyme glucose oxidase (GOx) and involved measurement of the reduction in oxygen tension for the reaction catalyzed by GOx (Gen-1) [23]. This was improved by wiring with redox mediators (Gen-2) [23,26] until finally direct electron transfer (DET) was demonstrated by Guiseppi-Elie et al. [27] allowing for direct communication of GOx with the transducer and biosensing system [23] respectively.

When discussing precisely how GQDs and CQDs may participate in biotransduction, there are some key aspects and specific properties to consider which make such systems ideal. First, they share comparable length scales to the biomolecular components with which they interface. This is important, since interfacing within the electron tunneling distance for electron transfer processes is vital [27,28]. Secondly, the Fermi energy level of electrons in the GQDs and CQDs may be tuned, allowing for them to participate in charge transfer processes with a wide variety of redox-active molecules or compounds [29]. Lastly, a wide reper-

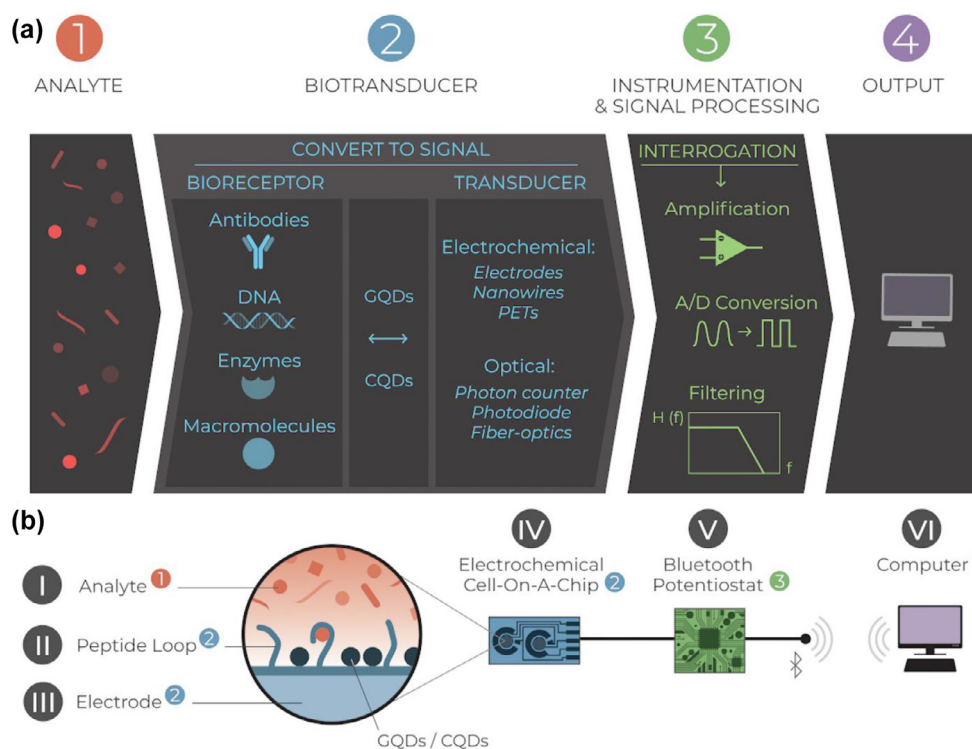


FIGURE 1

(a) Schematic outline of possible system configurations – with emphasis on graphene and carbon quantum dots interfacing with the biological recognition entity at the level of the biotransducer to form a nanobiosensor. Analyte detection is enabled by a biological recognition element co-joined with the physiochemical transducer. Depending on the engineered interface, physical detection method, and overall system integration, considerable signal processing may be required to achieve an actionable output. (b) An archetypical biosensor system showing the *biotransducer* which combines *biomolecular specificity* and the *physiochemical transducer*, the *instrumentation subsystem* and the *readout device*. (I) Matrix containing the analyte of interest and interfering substances, (II) the molecular recognition peptide loop with analyte capture at an engineered interface, (III) the electrochemical cell-on-a-chip comprising a microdisc array working electrode, a large area counter electrode and a reference electrode, (IV) a dual responsive biochip for multiplexed biotransduction, (V) a Bluetooth® dual potentiostat, and (VI) a computer-enabled base station for data analysis and presentation of actionable results. Adapted from [15].

toire of possible chemical modifications paired with overall excellent biocompatibility affords a large engineering space to be explored when developing physiologic biosensors. General classes of engineered biotransducer interfaces are presented in Table 2.

Photoluminescent and colorimetric biotransducers

Photoluminescent and colorimetric biosensors generally use the on–off quenching patterns afforded by confined nanomaterials [30–34]. Generally speaking, the bioreceptor or biological recognition entity is included in the system with the GQDs/CQDs, which present either in an “on” state (high fluorescence) or an “off” state (quenched fluorescence). Then, when the analyte is present, through either a binding event or a chemical reaction, the initial system is disrupted, which alters fluorescence. The benefit of photoluminescent and colorimetric biosensors is the relative ease of fabrication and their wide, general applicability to the detection of many different analytes, regardless of their chemical composition. This type of biotransducer also affords *in vivo* and *in vitro* sensing applications for real-time biological status monitoring both in laboratory and in the clinical settings. However, certain chemical reaction-associated systems are difficult to reverse once the GQDs/CQDs are chemically disrupted.

Additionally, the quantum yields for these nano-materials are still relatively low [1,34] and often have to be supplemented. Finally, the general, non-specific binding profile of GQDs and CQDs via π - π stacking is a double-edged sword; while it enables a wide variety of chemistries, off-target, interfering interactions are also more likely to occur.

Electrochemical biotransducers

Electrochemical biotransducers are powerful devices as these systems represent highly efficient means to detect, monitor, and quantify electron transfer and redox reactions to measure analytes. These systems integrate the electronic properties of GQDs and CQDs to improve electron transfer kinetics or kinetics at interfaces, anchor specific recognition molecules, or serve as carriers due to their surface area and curvatures. One of the exceptional benefits of electrochemical sensing is the wide variety of electrical techniques afforded by the system. Outputs may be measured amperometrically (current), potentiometrically (voltage), impedimetrically (AC impedance), and conductometrically (DC resistance), among others. Electrochemical sensing theoretically has an exceptional limit of detection, as the focus for analyte detection is electron transfer manifested as current, ideally mapping 1:1 electron tunneling and signal response. Direct elec-

TABLE 2

Biological transduction elements at a glance.

Molecular recognition component	Advantages	Disadvantages	Performance		References
			Limit of detection	Sensitivity	
Enzymes	- Direct integration of biological recognition facilitates detection via downstream products - Enzyme kinetics may be optimized - Wide variety of chemistries facilitate biosensor integration into optical, electrical, and combinations thereof - Often reversible chemistries	- Structural activity dependence complicates interfacial engineering - Stability and longevity concerns - Kinetically slow enzymes interfere with sensing capacity - Large macromolecular structure limits biotransducer designs	nM–μM May be measured kinetically via U/L ($\sim$ U/L)	1/μM–1/mM $\sim$ 1/(U/L)	[151,157,170,174,178]
Aptamers and Peptide Fragments	- Exceptional specificity for target analytes - Genomic DNA is an exceptionally stable biological macromolecule - Precise chemical control - May use DNA and RNA (aptamers) or peptides as needed - Low dimensions facilitate nanomaterial integration - Aptamers facilitate genomic amplification techniques	- Generation of appropriate aptamers is primarily via library trial and error - Affinity based aptameric biosensors may be reversible	fM–nM	1/fM–1/nM <i>In vivo</i> [162]: 1/mM	[178,179,195,196,200,201]
Antibodies	- Excellent specificity - Target recognition of structural motifs facilitates sensing of analyte conformation and modifications - Highest binding affinities of any common biological transducers - Sandwich design immunosensors expand sensing regimes and modalities	- Non-specific binding - Require considerable engineering for appropriate stability - Not conducive to electrical investigation and generally require secondary antibodies, impedance studies, and other supporting chemistries - Low molecular weight analytes cannot be detected	fg/mL–ng/mL Most biological analytes (proteins/nucleic acids) detected by antibodies have weights reported in kilodaltons (kDa; equivalent to g/mol). Thus, the molecular weights are in the thousands to hundreds of thousands of g/mol	1/(pg/mL)–1/(μg/mL)	[204,205,208,210,215,216,217,224]
Supramolecular/Macromolecules	- High stability as organic small molecules - Can participate in sophisticated chemistries with a capacity to retain chemical profile	- Less known - Reconstituting physiological chemistry and performance is non-trivial	Varying Porphyrinic sensors reported down to fM	1/pM–1/μM	[227,229,231,232]

tron transfer in electrochemical biosensors is itself a heavily investigated field and has been demonstrated using a wide variety of redox active enzymes with nanomaterials tailored to the interface [27,35–39]. Due to their length scale and electronic properties, GQDs and CQDs are exceptional candidates for interfacing with redox active biological materials. Another benefit of electrochemical sensing regimes is the wide repertoire of investigative techniques alongside commonly used amperometry and voltammetry, including electrochemical impedance spectroscopy and impedimetry [13,40]. There are several drawbacks to electrochemical sensing however, the most prominent being that without the presence of an electroactive species in solution, electrochemical detection may be difficult. Impedance studies address this weakness, usually by the addition of an electroactive species such as ferrocyanide(II) $[\text{Fe}(\text{CN})_6]^{4-}$ /ferricyanide(III) $[\text{Fe}(\text{CN})_6]^{3-}$, ferrocene/ferrocenium, and quinone/hydroquinone redox systems. Another weakness is the relative complexity these systems require for monitoring, and considerable interfacial engineering is needed to achieve the stability and reproducibility to adapt these systems to *in vitro* and *in vivo* applications. Lastly, one important consideration in such interfacial engineering is that biomolecular activity or structure is sufficiently preserved when the interface is introduced.

Photoelectrochemical and electrochemiluminescent biotransducers

Photoelectrochemistry (PEC) [41] and electrochemiluminescence (ECL) [42] link photonic excitation and emission with electronic transitions within a material. The two fields broadly describe current generated by light excitation, and light emission generated upon redox chemistry at an applied voltage, respectively. It is within PEC and ECL biosensing that the diverse properties of GQDs and CQDs are leveraged in ways that set them apart from other nanomaterials. Specifically, this type of interface engineering allows for precise design and coupling of materials to one another via conducting and valence band matching to form charge transfer pairs. The tunable nature of GQDs and CQDs further allows for tailor-made biotransduction systems that target the analyte and the energetic/photonic transitions needed to realize biotransduction. Despite the many degrees of freedom these systems afford, there are several drawbacks. These biotransducers are sophisticated systems and require precise engineering of the charge transfer interfaces and/or may require redox co-reactants. Additionally, specialized equipment and electrodes are required to design a PEC/ECL biotransducer, with such electrodes posing logistical burdens at integration as well as design and scale up limitations.

Characteristics of GQDs and CQDs

Compared to semiconductor quantum dots (QDs) [43], GQDs and CQDs display a variety of beneficial properties in addition to the traditional quantum confinement-based properties. They are highly water soluble owing to their surface chemical functional groups, display overall high *in vivo* biocompatibility [44–47], are easily chemically modified [2,10,44,46,47], and display native catalytic properties [48–55]. However, the source of their photoluminescent properties has not yet been entirely elucidated and is thought to be a compounding of defects, quantum confinement, chemical moieties, and edge effects [56]. Each is

discussed separately in detail below; Fig. 2 highlights several of their unique electronic properties.

A shared reason for using GQDs and CQDs in biosensing is the promising and almost unexpected biocompatibility they display [1]. GQDs and CQDs have been studied for biocompatibility in a host of ways ranging from *in vitro* [57–61] to *in vivo* studies with applications in imaging [44,46,58,59], photothermal therapy [62,63], and even native injection as a therapeutic for disaggregating pathological protein aggregates [64]. So far, the data indicates that, unlike other nanomaterials, GQDs and CQDs are relatively harmless to model cell and mouse systems. Preclinical studies of GQDs and CQDs are promising. It is likely that with additional safety and toxicity testing these nanomaterials can progress to the clinic.

GQDs

GQDs are confined graphene structures of sp^2 -hybridized carbon [4–7,65] with properties highly dependent on their synthesis method [1,2]. They are commonly edge-terminated by carboxylic acid groups [66] which confer their water solubility, however, other edge terminations, commonly amine [67–70] and boron [71,71–73], have also been reported. GQDs are more crystalline than their CQD counterparts and facilitate π - π stacking thanks to their planar structure. They have been synthesized in a wide variety of geometries, including circular, elliptical, and other polygonal shapes [2,74]. While quantum confinement experimentally begins to manifest in GQDs under 100 nm [7], most GQDs synthesized are within a range of 3–25 nm [66,75]. Chemical studies indicate that GQDs likely stack with one another in solution [76], although more recent work has reported single-layer GQDs and that different synthesis methods result in GQDs which may be more or less conducive to stacking [77]. Such systems are likely to display a high degree of structural dependency on dielectric constant, salt concentration and pH of the medium, salt concentration.

GQDs have a UV absorption peak and an additional shoulder peak at 260–320 nm and 270–390 nm respectively [2]. The former arises from π - π^* transitions in the GQD conjugated aromatic network while the latter is associated with $\text{n}-\pi^*$ transitions from the $\text{C}=\text{O}$ bonds present at the termini [75,78]. Each of these transitions may be altered upon chemical modification and shifted based on interactions with given matrix moieties. The surface moieties of GQDs confer responsiveness to different chemical environments, allowing for precise tuning of the photoluminescent response. GQDs display pH dependent quenching [4,79], which may be damped via surface passivation (binding a chemical moiety to the surface of the material) [80]. The photoluminescence has been reported to be tunable across many colors including blue [4,78,81,82], green [83–87], yellow [88–91], and red [92,93] via changing overall size, chemically bound/terminating/interacting groups, synthesis method, and the chemical environment [56]. GQDs have also been reported to display photoluminescent upconversion [80,94,95], a phenomenon where the emission wavelength is of a higher energy than the energy of the excitation wavelength. There are, notably, questions regarding the precise mechanism governing GQD upconversion as other photoluminescent mechanisms (such as two-photon

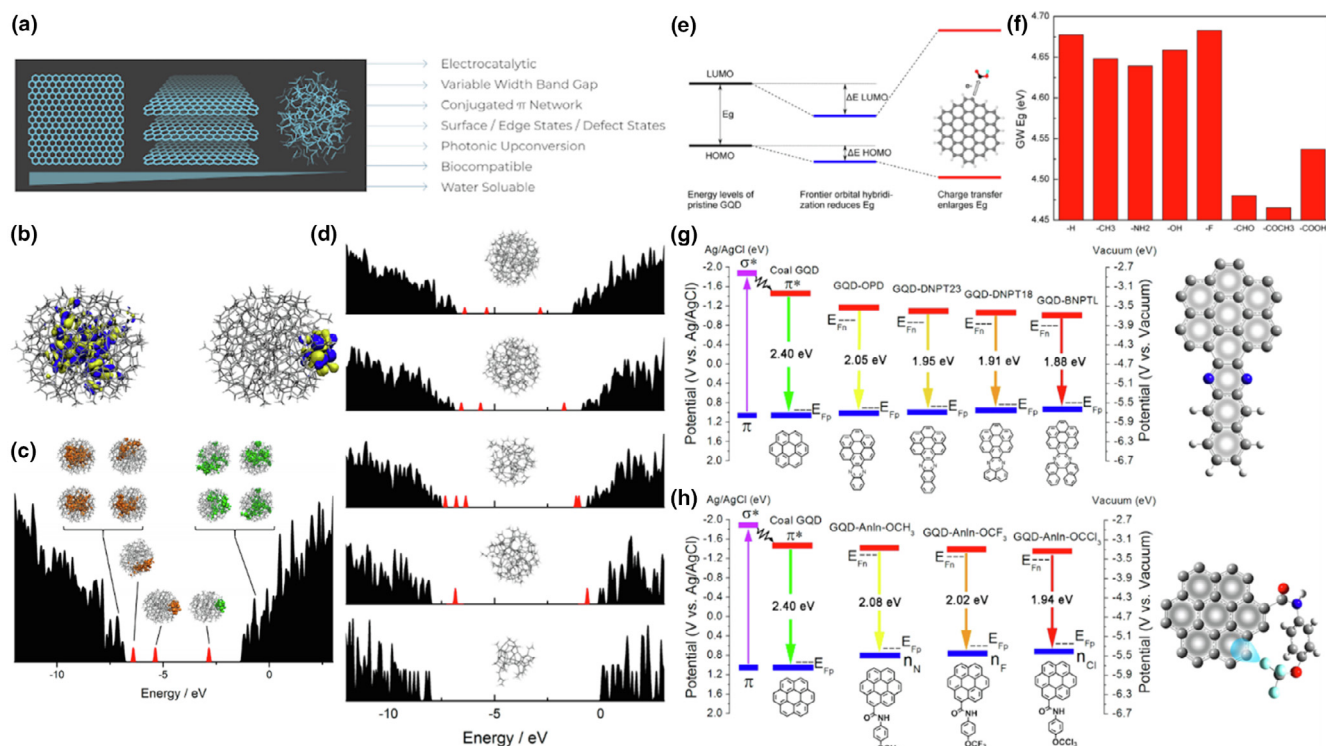


FIGURE 2

Electronic and band structure of graphene and carbon quantum dots. (a) Is a summary of key biosensor enabling properties of GQDs and CQDs, with the structures ordered by decreasing crystallinity (single graphene sheets being the highest and amorphous carbon dots being the lowest). (b) Illustrates examples of molecular orbitals which provide insight as to the band-like states and the surface states of CQDs. (c) and (d) Highlight the band gap of CQDs, with various contributing surface and band-like states, revealing a dependence on the size (all work from Margraf et al.). (e) Is a schematic reproduced from Li et al. which discusses how terminal groups participate in orbital hybridization as well as charge transfer processes, both of which alter the band gap of GQDs. (f) Highlights how each of the terminal groups alters GQD band gap (Li et al.). (g) and (h) Are work by which illustrate how π -orbital delocalization and various chemical groups influence the band gap of GQDs (Yan et al.). Current work highlights familiar patterns – such as increasing band gap with decreasing length scale – though the underlying etiology of such electronic states is considerably more complex than conventional quantum dots. (b), (c), and (d) Reproduced from Ref. [146], © 2015 American Chemical Society. (e) and (f) reproduced from Ref. [103], © 2015 American Chemical Society. (g) and (h) Reproduced from Ref. [105], © 2018 American Chemical Society.

absorption and second-harmonic generation) mimic the outcomes of upconversion [96,97].

One of the most important properties of GQDs is the ability to engineer the band gap and alter the highest occupied molecular orbital (HOMO) to lowest unoccupied molecular orbital (LUMO) electronic transitions. Theoretical framework for the properties manifested in GQDs stem from original work on graphene. Graphene itself has an infinite Bohr exciton radius (the distance between an electron and the paired electron hole) [98] which implies that any discrete graphene nanocompound will display confinement properties as the length scale of the material (nm) is on the order of or smaller than the Bohr exciton radius (∞) [99]. The physical scenario is, however, more sophisticated – as graphene itself does not have a bandgap and the multiple unique structural properties of GQDs can apparently influence the observed properties. Theoretical studies on GQDs note a strong dependence on size for influencing the band gap, with smaller materials (fewer C atoms) showing larger HOMO-LUMO transition energies. Strikingly, however, GQD's band gap dependence on length varies on the order of $1/L$, in contrast with the $1/L^2$ dependence exhibited by semiconductor QDs [99–101]. GQDs edge states also contribute non-trivially to the observed

HOMO-LUMO transitions, however, this is dependent on the density of states residing at the edge, which is further influenced by shape [99,101]. Ji et al. highlight these deviations in analysis of sub-2 nm GQDs by detailing that the conventional Dirac Fermion model for confinement does not fully recapitulate the properties of GQDs. However, incorporating precisely electron–electron and electron–hole interactions better reflects their physical properties [100]. Also, unique to GQDs are effects of chemically bound groups that introduce new electronic transitions [102–105]. Molecular modelling performed by Li et al. revealed that carbonyl functional moieties ($\text{C}=\text{O}$) influenced the HOMO-LUMO energy transitions more strongly than amine groups ($-\text{NH}_2$), although both affected the bandgap [103]. They attributed the π -electrons present in the $\text{C}=\text{O}$ bond to be the source of a prominent energetic downshift from extension of the conjugated π -network. Their work detailed a competing mechanism wherein functional groups would alter both HOMO-LUMO energies to reduce the band gap while their charge transfer properties would serve to increase it [103]. This type of band-gap engineering of GQDs to start with wide-gap bands and shrink them was experimentally realized by work by Hu et al. [104] and expanded upon later by Yan et al. [105], with

authors showing direct manipulation of the conjugated π -network delocalization and the presence of electron-donating groups.

GQDs stand out as biocompatible ECL and PEC nanomaterials. Li et al. first described this mechanism for GQDs where they observed ECL with an onset potential of -1.45 V in a pH 7.4 solution with $K_2S_2O_8$ [91]. Importantly, GQDs displayed no ECL on their own, requiring $K_2S_2O_8$ as a co-reactant. Using this finding, they proposed a mechanism involving reduction of both species and an electron transfer annihilation that resulted in the generation of an emitted photon from the excited-state GQD post-annihilation [91]. This general reaction schematic of a redox active co-reactant is a general pattern in GQD ECL. Lu et al., in later work, reported an ECL onset potential of 0.4 V while using H_2O_2 as a redox co-reactant [81]. The exceptional electrical properties and length scale of GQDs have also recently facilitated DET in biotransducer development [54,106].

Another unique feature of GQDs is their intrinsic magnetic properties, which are strongly dependent on size and shape [107]. These properties were posited and explored by several computational studies [108–112] and more recently experimentally elucidated [113]. There are two general sources of GQD magnetism parsed out, zero-energy states which reside at the Dirac points and confer temperature-dependent paramagnetism, and dispersed edge states which impart temperature-independent diamagnetism [108]. Experimentally, however, obtaining intrinsically magnetic, oxide terminated GQDs is difficult [107]. A report by Sun et al. highlights that while innately paramagnetic GQDs are chemically feasible, most GQDs synthesized are not appreciably magnetic [113]. They proposed that most of the magnetism originated at zigzag edges that were passivated by hydroxyl groups. Uniquely doped GQDs have additionally been synthesized (for example, boron [114] and fluoride [115]) which were reported to have intrinsic paramagnetism, showing that additional doping of the GQDs can influence their magnetic properties.

A property which facilitates unique integration into increasingly sophisticated biosensing systems is the innate enzyme mimicry that GQDs possess [116], that is, the capacity to act as nanozymes. Shi et al. pioneered this field in 2011 by characterizing the enzyme mimicry exhibited by GQDs [117]. Described as a “peroxidase-like” activity, they attributed the observation to the intrinsic ability of GQDs to reduce H_2O_2 by acting as an electron-dense donor. They also noted the importance of the carbonyl and carboxylic acid groups in such mechanisms, and further discussed that GQDs potentially possessed stability that surpassed enzymes they mimicked. Additionally, and much like enzymes, kinetics were influenced by pH, temperature and concentration. They concluded their communication by developing a biosensor that detected glucose in solution. In parallel, Wang et al. described a similar system and made special note that temperature and kinetic rate were positively correlated [118]. Following these works, Sun et al. confirmed the necessity of the carbonyl groups for catalytic activity and the carboxylic acid groups as binding sites by selectively deactivating these groups individually [55]. GQDs have been modified and doped considerably to improve the reaction kinetics and increase stability and activity in complex media [116,119–122]. Coupling the nanozyme

mimicry to biological recognition agents has proven to be an exciting and expanding area of biosensor development. In addition to being nanozymes, GQDs are electro- and photocatalytic and are able to catalyze, among other reactions, oxygen reduction (electrocatalytic) and hydrolysis (photocatalysis) [120].

CQDs

Compared to GQDs, CQDs display significantly less crystallinity, with x-ray diffraction patterns indicating the existence of disordered carbon [123] and structural patterns akin to that of amorphous carbon. Most commonly, CQDs are <10 nm [1,2,123,124] and are comprised of a varying mixture of sp^2 - and sp^3 -hybridized carbon [52,123]. CQDs also possess surface carboxylic acid groups and surface amine groups which impart water solubility [52,123] but have also been synthesized as boron-doped [72,124–126] and sulfur-doped [127].

One of the defining characteristics of CQDs is their photoluminescent properties. CQDs have a strong absorbance band in the UV range (260–320 nm [2,75]) and have been tuned across the visible spectrum from blue to red [123,128–132], with properties dependent on the chemical environment such as pH [133,134]. The source of this photoluminescence is not entirely clear. Chemical modeling of CQDs points to defects and surface states that impart luminescent properties, however their amorphous structure complicates such endeavors [29]. CQD properties are also highly dependent on their synthesis methods; different precursors and methods yield variations in CQDs chemically and structurally [1,2,29,52,75], but generally function as both strong electron acceptors and donors [135–137]. CQDs also have numerous reports in literature discussing the observation of photoluminescent upconversion [138–141].

Despite their earlier discovery, the theoretical work describing the electronic properties of CQDs is only recently beginning to be explored. Like their GQD counterparts, size and surface group modification influence their properties similarly [29,56], although these processes are less precise to describe. There is a clear connection between the surface states of CQDs, the presence of emissive traps (intermediate energy states which “trap” excited electrons and result in decreased fluorescence), and surface passivation to damp the influence of these traps [142–145]. Work by Margraf et al. using semiempirical molecular orbital theory placed special emphasis on the topology of CQDs when determining band gap, with sp^3 carbon atoms only participating minimally [29,146]. In their report, they relay that CQDs, while displaying confinement dependency, have generally convoluted sources of their band gap energies which depend on the degree of conjugation, with less emphasis on the atomic hybridization itself and the presence of dopants/function groups, setting them apart from GQDs. In amorphous CQDs, surface electronic states are strongly responsible for photoluminescent/electronic properties [147]. There are also distinct regimes of electronic deactivation pathways related to CQD photoluminescence, a fast pathway within the CQD core related to the sp^2 -hybridized carbons and a slow process related to the density of surface states present on the amorphous CQD [29,147].

CQDs are less conducive to electrochemical systems than are GQDs, due to their shape and amorphous nature, which complicate interfacing them directly with biological systems. Neverthe-

less, they share similar properties with GQDs and also display the same ECL mechanism. Indeed, discovery of this mechanism predates GQDs [131,148] and the initial ECL groundwork for carbon nanodots was first established with CQDs.

The magnetic properties of CQDs are less studied than those of GQDs. This may be due to the fact that CQDs lack definite edge structures and patterns which simplified the study of magnetic properties in GQDs. Nonetheless, there have been some reports in literature of magnetic CQDs that have been doped and reported to exhibit intrinsic magnetism [149,150].

Much like GQDs, CQDs possess enzymatic activity and share many of the same chemical and molecular mechanisms which impart the catalytic property [116,120,121]. There are differences between the two materials kinetically – GQDs have been reported to possess higher binding affinities, though CQDs are reported to have higher maximum kinetic rates [121]. This could be due to differences in surface area, chemical doping levels, terminal groups, and/or dependence on system parameters. However, overall, this is a property shared between the two nanomaterials. And, like GQDs, CQDs are also electro- and photocatalytically active [120].

Applications of GQDs and CQDs to biosensing

Enzymatic biosensing

One of the classic biosensing schemes is the use of immobilized enzymes as the biological recognition element. Enzymes have evolved to bind with high affinity to the target analyte and catalytically convert it to a measurable product and were incorporated into the first, elegant assays using nature's machinery for detection and biotransduction. They also represent a considerably large portion of clinically employed sensing (e.g. blood glucose monitoring, ELISAs). In this section, various interfacial endeavors in the field are reviewed and discussed. Also included in this section are applications of the peroxidase-mimicking capacity of GQDs and CQDs as they are incorporated into enzymatic biotransducers. The various apparent strengths are the reusability, breadth of chemistries, and ease of use. Drawbacks stem from the dependency on enzymatic conformation for activity – which can be adversely altered in sub-optimal conditions – as well as the large sizes of proteins to incorporate into the device interface. Accessing the active sites of proteins becomes complicated for those buried deep within the enzyme.

Photoluminescent and colorimetric biosensors

One of the most direct forays into enzymatic GQD/CQD sensing is via fluorescent interactions. Li et al. reported a fluorescent GQD probe designed to detect hydroquinone in solution [151]. Horseradish peroxidase (HRP) catalyzed the conversion of hydroquinone to 1,4-benzoquinone, which served as an electron acceptor that quenched the GQDs. Xiaoyan et al. reported GQD usage in their biosensing system to increase the catalytic activity of HRP by roughly $1.9\times$ [152], which they attributed to conformational changes induced by the GQD/HRP interaction. In the presence of H_2O_2 , HRP and GQDs would catalyze the oxidation of tetramethylbenzidine, which then quenched the GQDs. They then used this to probe for H_2O_2 levels in solution, reporting a very wide linear detection range from 2 nM to 200 μM and a limit of detection (LOD) of 0.2 nM. In the presence

of H_2O_2 , HRP and GQDs would catalyze the oxidation of tetramethylbenzidine, which then quenched the GQDs. Caballero-Diaz et al. described a colorimetric sensor which used acetylcholinesterase (AChE) as the biological component to detect organophosphates [153]. The products of neurotransmitter decomposition natively quench GQDs, however, in the presence of neurotoxic organophosphates (in this case, fenoxycarb the carbamate insect growth regulator), AChE was inhibited and the GQDs retained their fluorescence. Sahub et al. and Li et al. performed similar work but integrated, respectively, choline oxidase and a colorimetric component into the system [154,155]. A similar regime was explored for α -glucosidase activity by Kong et al. where the byproducts of said enzyme (4-nitrophenol) quenched CQDs natively [156]. They used their system to quantify both enzyme activity and inhibitor concentration. Huang et al. reported a biosensor that used alkaline phosphatase to catalyze formation of phenol, which quenched GQDs [157]. Like previous studies, they also used the system to study inhibitor concentrations. A ratiometric system for glucose biosensing was developed by Cho et al. [158] where they used CQDs in conjunction with rhodamine 6G. They observed a color transition from blue to green as glucose concentration increased. Their system had a wide linear range from 0.1 to 500 μM and a low LOD of 40 nM. A metabolite-based sensor was developed by Li et al. which used tyramine functionalized GQDs as a turn-off sensor when H_2O_2 was present [159]. Their work is highlighted in Fig. 3 in detail, where their proposed schematic of linking GQD quenching to an oxidoreductase is discussed and the adaptability is stressed. This sensor could be coupled to many different metabolically relevant enzymes, with cross-linked GQDs (and quenching) used as the universal readout.

One of the major applications for GQDs and CQDs involves their excellent biocompatibility, and thus some foray into *in vivo* and *in vitro* biosensing with fluorescent probes would prove to be an incredibly useful tool for biological endeavor. Tang et al. addressed this underlying limitation of many current fluorometric GQD/CQD-based biosensors by directly conjugating tyrosinase to CQDs to yield a dopamine bioprobe [160]. While the overall system was comparable to the previously discussed systems, the ability to directly conjugate the entire sensing system into a single unit is a step forward in expanding the versatility of such nano-enabled biotransducers. Lastly, some unique proteins have been explored, such as hemoglobin [161,162], uricase [163], and urease [164].

One of the more recent and unique applications in biosensor development came from coupling the intrinsic catalytic capacity of GQDs and CQDs with other biological processes to monitor output. Most commonly in literature, this involves coupling the peroxidase-like properties of the nanomaterials with an oxidoreductase, such as performed in the first reports of peroxidase mimicry [117,118]. Other recent work has focused on improving the platform, though many of the patterns remain similar. Zhou et al. reported a system which took unique advantage of the catalytic properties of GQDs to catalyze the oxidation of tetramethylbenzidine to integrate pyranose oxidase for a sensing system to detect 1,5-anhydroglucitol [165]. Lin et al. report a glucose biosensor by coupling GOx to the catalytic oxidation of 3,3',5,5'-tetramethylbenzidine [166]. In this scheme, the GQDs

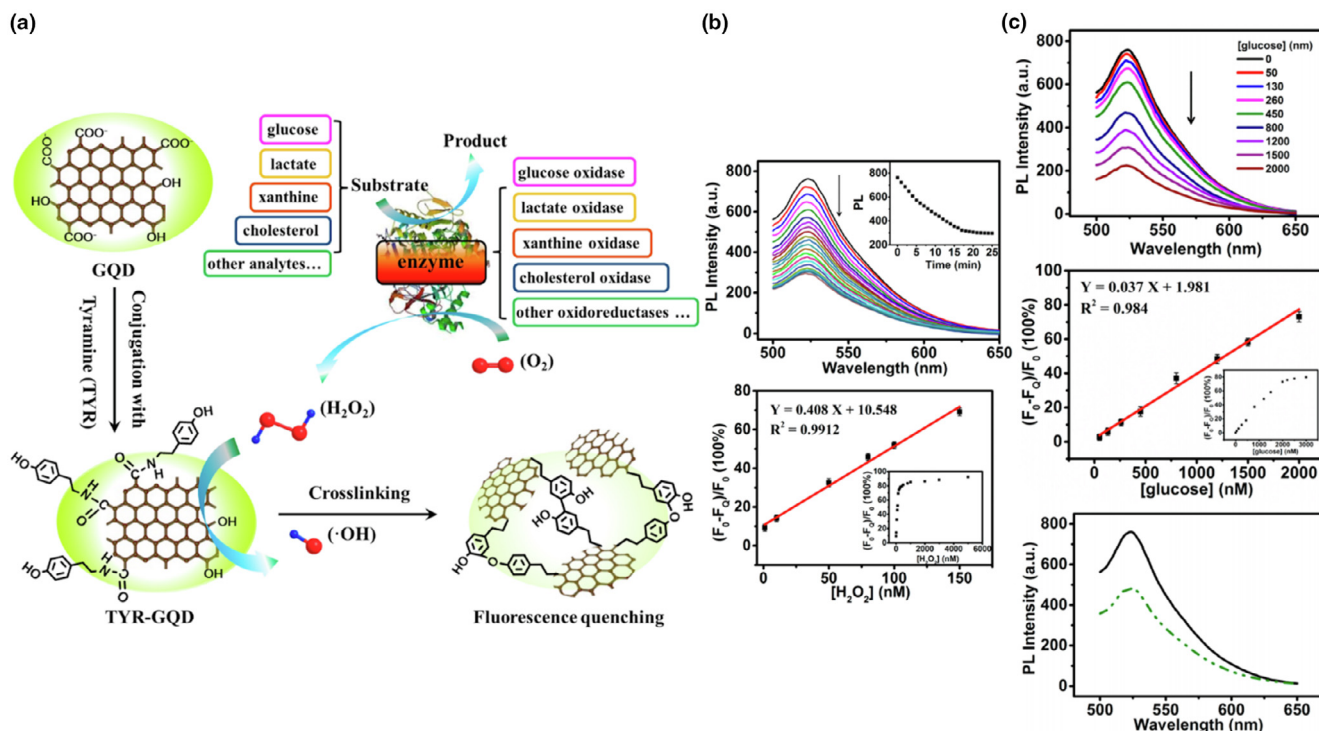


FIGURE 3

Li et al. developed a general profiling method based on the photoluminescent properties of QGDs, which is detailed in (a). Based on the metabolite of interest, an oxidoreductase enzyme which catalyzed the oxidation of the analyte and formation of H_2O_2 was selected, some examples of which are listed. The response factor to this catalytic event is the oxidative cross-linking of tyramine-conjugated QGDs in the presence of H_2O_2 , quenching the original photoluminescent tyramine-QGD conjugates. The generality of this system is its power, as any enzyme which produces H_2O_2 is potentially compatible. (b) Highlights time dependent cross-linking of the tyramine-QGDs in the presence of H_2O_2 (top) and the response to different concentrations (bottom). (c) Is a representative response which uses GOx, showing the luminescent response (top), the linear response range (middle), and the response in serum (bottom). Reproduced from reference [159], © 2016 American Chemical Society.

themselves served as the catalyst for the colorimetric assay – GOx only converted glucose to gluconic acid and released H_2O_2 . Wang et al. reported an improvement to this scheme by incorporating GQDs enriched in carbonyl and carboxylic acid groups [167]. Their chemical advancement yielded strong performance, with their report showing an LOD of $0.2 \mu\text{M}$ and good functionality in blood samples. Nanowire support has also been employed in this glucose reaction scheme. Zhong et al. used nanowire etching in the presence of H_2O_2 , GQDs, and iodine to reduce the absorbance of the nanowires and detect glucose [168]. This application was unique in its endpoint as it did not employ 3,3',5,5'-tetramethylbenzidine. Honarasa et al. employed V_2O_5 nanowires as a scaffolding for GQDs [169] to improve overall reaction kinetics and activity.

Electrochemical biosensors

Owing to their high surface to volume ratio and adsorptive chemistries, GQDs and CQDs offer potential as simple immobilizing agents for developing enzymatic biosensors. The benefits offered by these materials are due to their strong electronic properties, which provide minimal passivation while acting as electrode scaffolds. Vasilescu et al. used these properties to develop a molybdenum disulphide (MoS_2)/GQD biotransducer onto which they then drop-casted laccase to successfully fabricate a polyphenol index biosensor [170]. The caffeic acid sensor they developed

was reported to have a linear range of 380 nM–100 μ M with an LOD of 320 nM. Of note in their voltammetric studies was that the laccase retained a similar response across the various developed electrode formulations, however, the MoS₂/GQD electrode had vastly improved electron transfer kinetics.

Direct electron transfer is an ideal approach for facilitating electrochemical biosensing using redox active enzymes. This is one of the most powerful ways that GQDs and CQDs enable biosensing. Razmi et al. reported interfacing GQDs with GOx to develop an electrochemical biosensor [171], which is discussed in Fig. 4. A similar schematic was reported by Muthurasu et al. who interfaced HRP with GQDs for a H_2O_2 biosensor [172]. Their electrochemical biosensor displayed two linear ranges, one in the μM and another in the mM regime, with an overall LOD extending to 530 nM. Wang et al. reported a system which improved immobilization strategies by using CoFe as charged scaffolds to facilitate HRP attachment and a direct electrical connection with CQDs [173]. Interestingly, DET was enhanced by the presence of CQDs, although it was not absent from other control schematics. Zhao et al. reported a study of DET facilitated by CQDs for both GOx and bilirubin oxidase [174]. Work by Gupta et al. drop-casted GQDs with GOx to develop a glucose biotransducer but extended their work beyond glucose sensing to explore other analytes, engineering the interface between redox enzymes and GQDs using GOx, HRP, cytochrome-C, and myoglobin, though

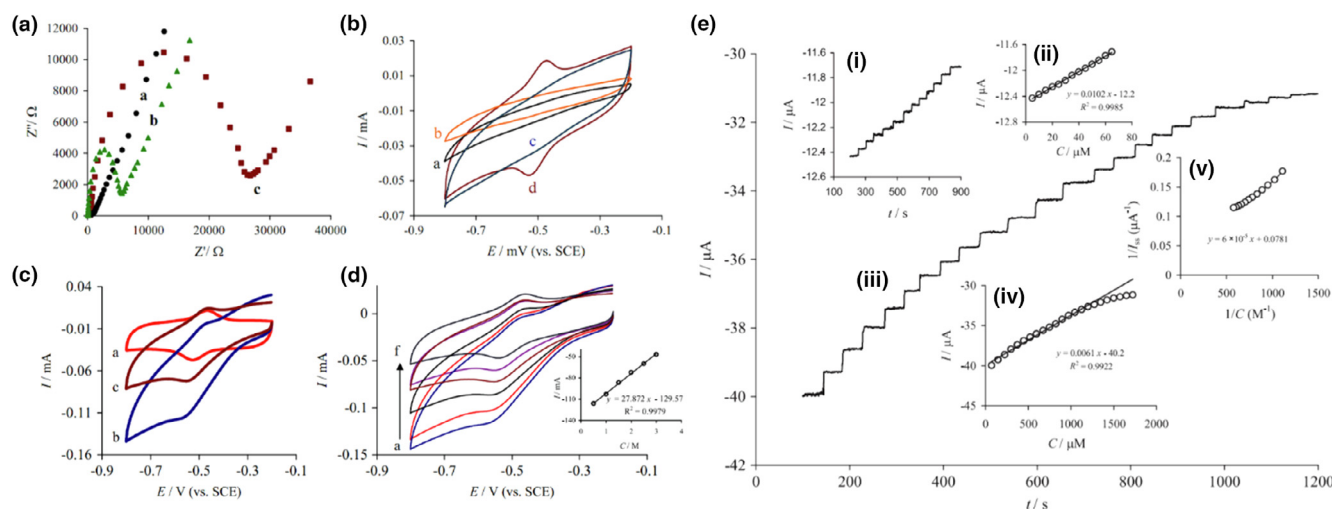


FIGURE 4

Direct electron transfer of GOx as facilitated by GQDs. Razmi and Mohammad-Rezaei immobilized GOx onto a GQD modified electrode – which is characterized in (a), noting an increase in charge transfer resistance, R_{CT} , upon GQD functionalization and a dramatic increase upon enzyme immobilization. (b) Details characteristic cyclic voltammograms (CVs) of the modified electrode. Note, on the red curve, (d), the appearance of a quasi-reversible set of peaks. (c) Highlights CV evolution in the presence of glucose, with (d) detailing mM addition of glucose and changes in the CVs. (e) Details amperometric response at constant potential of -0.42 V in 0.1 M PBS (pH 7.4). In this schematic (b) and (d) are the standard curves of (a) and (c) respectively, exploring the dynamic range and linearity of response. K_M was determined via a Lineweaver-Burk plot, which was reported to be 0.76 mM. Reproduced from Ref. [171], © 2013 Elsevier.

other enzymes had broader redox peaks [54]. Baluta et al. reported using laccase to detect epinephrine by interfacing GQDs with the enzyme and observing DET [175].

Chemiluminescent biosensors

Hassanzadeh and Khataee reported that a mixture of MoS_2 QDs and GQDs resulted in a strong enhancing effect on the chemiluminescent reaction between H_2O_2 , which they then coupled to cholesterol oxidase to devise a cholesterol biosensor [176]. Their system had a wide linear range, from 80 nM to 300 μM , and an LOD of 35 nM. This system represents one of the more novel studies which couples a chemiluminescent reaction to that of the catalytic activity of GQDs, which may be a route to develop mediator based colorimetric biosensors using CQDs and GQDs that further increase the dynamic range and LOD via a secondary, more powerful/stable reaction.

Photoelectrochemical biosensors

Enzymatic photoelectrochemical biosensors are also only recently being reported. Recently, Cheng et al. reported a photoelectrochemical system which uses AChE to catalyze the conversion of acetylcholine to thiocholine [177]. There are several notable characteristics about their system. Visible light was the excitation driving force for the system and CQDs were used as the photocurrent potentiator. The presence of thiocholine provided an electron donor and increased the photocurrent output for the system, which was reversible in the presence of AChE inhibitors. They reported a linear detection range for inhibitor concentration of 1 ng/mL– 1.5 $\mu\text{g/mL}$ with an LOD of 70 pg/mL.

Summary

Overall, enzymatic sensing in literature with CQDs and GQDs is relatively streamlined. Most fluorescent sensors are aqueous, 1-

pot chemistries which present difficulty in higher order translations. Electrochemical sensing is commonly performed using drop-casted, redox active enzymes. However excellent progress is being made in DET-enabled sensing using CQDs and GQDs. Enzymatic biosensors which integrate the optical properties of GQDs and CQDs with their electrical properties are currently rare in literature – but several avenues are feasible to explore. In consideration that such current work involves aqueous chemistry and has potential applications in drug screening, a first-order upscaling to a lab-on-a-chip microfluidic system for high throughput, colorimetric screening would allow for parallelization of the presented systems and translate these technologies past isolated research endeavors. See Table A1 for a technical summary.

Aptamer, peptide, and genomic-based biosensing

Aptamers are 20–80 nucleotide, single stranded DNA or RNA sequences (oligonucleotides) which have a unique affinity for a target analyte. Peptide fragments can behave similarly, with the obvious difference lying in the chemical structure, the latter being comprised of amino acids instead of nucleic acids. Aptamers and peptide fragments are generally made via brute force chemical library searches, but do occur naturally, most commonly in bacteria (RNA riboswitches). There are many benefits of using aptamers – they are stable, specific, small, and support a wide variety of chemistries. However, the main drawback is that without prior availability of an appropriate aptamer for the analyte, developing a suitable construct is non-trivial. In this section, recent advances for aptamer-, peptide-, and other genomic-based biosensors are covered; these are included together due to many common patterns seen in biotransducer development using these technologies. One unique property that draws a distinction between aptamers/genomic sensing

TABLE A1

Enzymatic biosensing at a glance.

Authors	Biorecognition	Analyte	Linear range	Limit of detection	Notes	Refs.
<i>Photoluminescent and colorimetric biosensors</i>						
Li et al.	Horseradish Peroxidase	Hydroquinone	0.24–9.6 μM	84 nM	On/Off Sensor	[151]
Xiaoyan et al.	Horseradish Peroxidase	H_2O_2	2 nM–200 μM	0.2 nM	On/Off Sensor	[152]
Caballero-Díaz et al.	Acetylcholinesterase	Fenoxycarb	6–70 μM	3.15 μM	Inhibitor Based	[153]
Sahub et al.	Choline Oxidase	Dichlorvos	0.45–45.25 μM	0.172 ppm/0.778 μM	Inhibitor Based	[154]
Li et al.	Acetylcholinesterase	Paraxon	0.001–1.0 $\mu\text{g/mL}$	0.4 ng/mL	Ratiometric	[155]
Kong et al.	α -Glucosidase	Activity; Acarbose	0–10 U/mL; 100 nM–1 mM	0.01 U/mL; 10 nM	Inhibitor Based	[156]
Huang et al.	Alkaline Phosphatase	Activity; Na_3VO_4	0.02–0.08 U/mL; 0–1 mM	0.008 U/mL; –	Inhibitor Based	[157]
Cho et al.	Glucose Oxidase	Glucose	0.1–500 μM	40 nM	Ratiometric	[158]
Li et al.	Oxidoreductases	Variable	Variable	Variable	General System	[159]
Tang et al.	Tyrosinase	Dopamine	0.1–60 μM	60 nM	Conjugated	[160]
Bui and Park	Hemoglobin	Cholesterol	0–800 μM	56 μM	On/Off Sensor	[161]
Bhunia et al.	Hemoglobin	H_2O_2	0–100 mM	–	On/Off Sensor	[162]
Kong et al.	Uricase	Uric Acid	5–500 μM	2 μM	On/Off Sensor	[163]
Shao et al.	Urease	Urea	0.1–100 mM	0.01 mM	On/Off Sensor	[164]
Shi et al.	Glucose Oxidase	Glucose	1 μM –0.5 mM	0.4 μM	Nanozyme	[117]
Wang et al.	Glucose Oxidase	Glucose	0–100 μM	20 μM	Nanozyme	[118]
Zhou et al.	Pyranose Oxidase	1,5-Anhydroglucitol	20–100 $\mu\text{g/mL}$	144 ng/mL	Nanozyme	[165]
Lin et al.	Glucose Oxidase	Glucose	25–375 μM	5.3 μM	Nanozyme	[166]
Wang et al.	Glucose Oxidase	Glucose	0.2–50 μM	0.2 μM	Nanozyme	[167]
Zhong et al.	Glucose Oxidase	Glucose	0.01–2 mM	3 μM	Nanozyme	[168]
Honarasa et al.	Glucose Oxidase	Glucose	0.7–300 μM	0.7 μM	Nanozyme	[169]
<i>Electrochemical biosensors</i>						
Vasilescu et al.	Laccase	Caffeic Acid	380 nM–100 μM	320 nM	Amperometric	[170]
Razmi et al.	Glucose Oxidase	Glucose	5–60 μM	1.73 μM	Amperometric/DET	[171]
Muthurasu et al.	Horseradish Peroxidase	H_2O_2	0.1–1.3 mM; 1.7–2.6 mM	530 nM	Amperometric/DET	[172]
Wang et al.	Horseradish Peroxidase	H_2O_2	0.1–23.1 μM	40 nM	Amperometric/DET	[173]
Zhao et al.	Glucose Oxidase	Glucose	0–64 mM	1.07 μM	Amperometric/DET	[174]
Gupta et al.	Many	Many	10 μM –3 mM (Glucose)	1.35 μM	Amperometric/DET	[175]
Baluta et al.	Laccase	Epinephrine	1–120 μM	83 nM	Amperometric/DET	[176]
<i>Chemiluminescent biosensors</i>						
Hassanzadeh et al.	Cholesterol Oxidase	Cholesterol	80 nM–30 μM	35 nM	Nanozyme	[177]
<i>Photoelectrochemical biosensors</i>						
Cheng et al.	Acetylcholinesterase	Acetylcholine	1 ng/mL–1.5 $\mu\text{g/mL}$	70 pg/mL	Visible Light	[178]

and peptide sensing is that the former can employ DNA/RNA polymerase machinery to amplify nucleic acids – solidifying a unique niche for these sensors and opening up applications to molecular genetics.

Photoluminescent and colorimetric biosensors

Loo et al. reported a fluorescent ssDNA probe/CQD sensor for complimentary DNA strands in solution [178]. In this scheme, the π - π stacking from the DNA base pairs and CQD conjugated π -systems facilitated Fluorescence Resonance Energy Transfer (FRET) quenching. When the ssDNA hybridized with the target, this π -system interaction was lost and electrostatic repulsion dominated, restoring the fluorescence of the DNA probe. Shi et al. reported a FRET biosensor for detecting the *Staphylococcus aureus* mecA gene [179]. In their work, GQDs and Au nanoparticles were conjugated to ssDNA sequences that would hybridize in the presence of the mecA sequence, forming a FRET pair. Liang et al. designed a ratiometric dsDNA biosensor using CQDs and CdTe quantum dots [180]. CQDs were used as the ratiometric constant and CdTe dots used as the quenched readout, with the presence of dsDNA restoring fluorescence by interacting with the quenching agent, mitoxantrone. Wang et al. designed a set of

hairpin DNA loops and functionalized two sets of GQDs, each one with one of the probes [181]. In the presence of a target DNA strand (p53 mutant strands) the hairpins would hybridize with the strand to form a large meshwork of DNA/GQDs. They reported an exceptional 0.8 pM LOD and two discrete linear ranges namely 1.0 pM–1.5 nM and 1.5–50 nM respectively.

A hybrid-like system was reported by Kermani et al. for DNA methyltransferase activity [182]. In their work, they functionalized GQDs with a hybridized DNA strand that quenched the particles and contained the recognition site for both the DNA methyltransferase (M.SssI) and a DNA cutting enzyme (endonuclease: HpaII). If the DNA methyltransferase was present, it would methylate the DNA at its recognition site, and prevent endonuclease-mediated cutting. Thus, if the methyltransferase were present, fluorescence would remain quenched, whereas without the methyltransferase, the endonuclease would cut the DNA and restore the GQD fluorescence.

Aptamers and aptasensors are also present in literature and occupy a similar domain as genomic sensing. Aptamers offer many of the benefits of hybridization-based biosensors. One of the early aptasensors developed using GQDs/CQDs was reported by Wang et al. [183] using CQDs as FRET acceptors alongside

phosphors modified with a thrombin aptamer oligonucleotide. The oligonucleotide associated with the CQDs via π - π interactions, however, in the presence of thrombin, the aptamer preferentially bound to the analyte, releasing the CQD from the complex and restoring fluorescence of the phosphors. Their system had excellent specificity and displayed a linear response from 0.5 to 20 nM with an LOD of 180 pM. A few years later, Zhu et al. reported a dopamine aptasensor which used a competitive binding scheme wherein CQDs were conjugated to a DNA aptamer via carbodiimide chemistry [184]. CQDs were then loaded onto nanographite via π - π stacking and were displaced in the presence of dopamine, leading to a turn-on fluorescent biosensor. They reported a linear range of 0.10–5 nM, with an LOD of 55 pM. Shi et al. applied the competitive binding design to detect cancer markers [185]. In their work, the scaffold were MoS₂ nanosheets which supported adsorbed GQDs functionalized with poly(ethylene glycol) and an aptamer specific to epithelial cell adhesion molecule (EpCAM). In the presence of EpCAM, the GQDs dissociated from the nanosheet and then fluoresced.

Luo et al. presented a sophisticated system for the aptasensing of adenosine triphosphate (ATP) in solution using CQDs and graphene oxide (GO) as the scaffold [186]. Their system operated on a strand displacement principle; in their approach, the CQDs were conjugated to a signaling strand which itself could bind to the aptasensing strand and a helper strand. Binding prevented association with the GO, and the CQDs then fluoresced. In the presence of ATP, the aptamer changed conformation and released the helper strand to recruit a fuel strand, which removed the entire aptasensing strand complex from the CQD, allowing them to associate with the GO and be quenched. Recently, Zhang et al. described development of a GQD aptasensor capable of intracellular signaling (Fig. 5c–e) [187]. While they reported a relatively high LOD for an aptasensing system (0.27 mM), their work is significant as it applies conjugated FRET aptasensing systems to *in vitro* biological systems, one of the great strengths of GQDs and CQDs.

Two peptide biosensors were also reported and used to detect trypsin [188,189]. In both systems, the GQDs were quenched in some way, but the presence of trypsin cleaved either the quenching protein (cytochrome C in Li et al.) or cleaved peptide fragments to scavenge quenching ions (Su et al.). Wang et al. reported a different peptide biosensor which was used to detect casein kinase II [190]. In their work, they conjugated a peptide to GQDs, which could be phosphorylated in the presence of the kinase, imparting a negative charge. Then, upon addition of the positive cation, the GQD/peptide complexes aggregated, resulting in quenching. A linear range of 0.1–1.0 U/mL and a low LOD of 0.025 U/mL we reported.

Electrochemical biosensors

One of the early electrochemical aptasensing works was reported by Zhao et al. where they modified a graphite electrode with GQDs bound to ssDNA [191]. The presence of the ssDNA bound to the GQD-modified electrode blocked the electrochemical ferri-/ferrocyanide redox reaction. However, when the complementary DNA strand or thrombin was introduced into solution, the surface bound passivating DNA was removed and the electro-

chemical response was recovered. In the following years, Peng et al. reported an electrochemical recasting of work performed by Kermani et al. that same year [192]. They used a signaling ssDNA strand immobilized onto a gold electrode to probe for an analyte ssDNA strand. After an incubation aimed to induce hybridization, the electrode was blocked and the strands were treated with the CpG methyltransferase, M.SssI, to induce methylation and then HpaII to cleave any un-methylated DNA strands. Afterwards, GQDs were added and conjugated to the dsDNA strands still present on the electrode surface. Then, HRP was added to catalyze the oxidation of tetramethylbenzidine, which was subsequently detected electrochemically. Rezaei et al. reported a combined approach for aptasensing by using differential pulse voltammetry and electrochemical impedance spectroscopy to detect lysozyme (Fig. 5a–b) [193]. For both sensing methods, authors reported excellent ranges of detection, 20 fM–10 nM and 10 fM–100 nM, and very low LODs, 3.7 fM for DPV and 1.9 fM for EIS respectively.

Another notable electrochemical aptasensor for carcinoembryonic antigen (CEA) detection was developed by Huang et al. who incorporated DNAzyme-based signal amplification [194]. They used a GQD/naion/ionic liquid modified electrode, wherein the detection scheme was comprised of a DNA hairpin complex, which included the CEA aptamer oligonucleotide conjugated to the DNAzyme sequence. In the presence of CEA, the hairpin loop was broken and the DNAzyme was exposed and catalyzed the cleavage of many methylene blue/dsDNA substrates in the presence of Pb²⁺. The newly cleaved methylene blue/ssDNA complexes became bound to the electrode, eliciting a response. An auxiliary DNA strand was used to prevent long-term fouling. They reported a linear range of 0.5 fg/mL–0.5 ng/mL and an overall extremely low LOD of 0.34 fg/mL.

Hu et al. reports an RNA biosensor to detect miRNA-155 which operated on a similar axis as the electrochemical methyltransferase sensor described by Peng et al [195]. In their system, the probe DNA strands bound to a gold electrode would only hybridize with the target DNA strand if miRNA-155 was present. They bound GQDs and HRP to the hybridized strands and electrochemically detected the catalysis product as a signal amplification method. Their system had a linear range from 1 fM to 100 pM, and an excellent LOD of 0.14 fM.

Electrochemiluminescent biosensors

Early work on ECL biosensing with these materials was performed by Lu et al. where they modified the surface of a gold electrode with immobilized ssDNA and conjugated a complementary strand to GQD/SiO₂ complexes which hybridized in the presence of ATP (Fig. 6c–e) [81]. ECL intensities served as the readout for the ATP concentration. An amplification-based sensor was reported by Zhang et al. to detect miRNA [196]. In their system, authors loaded GQDs onto an organic conductor molecule and then doped that structure with core-shell Fe₃O₄-Au nanoparticles. Subsequently, they cast this material onto an electrode to attain a solid-state GQD electrode with ECL capacity with S₂O₈²⁻ as the co-reactant. They then built a sequential array system. First, the electrode was doped with hairpin DNA sequences aimed to bind to a specific helper DNA strand. The electrode was then blocked and helper ssDNA strands were intro-

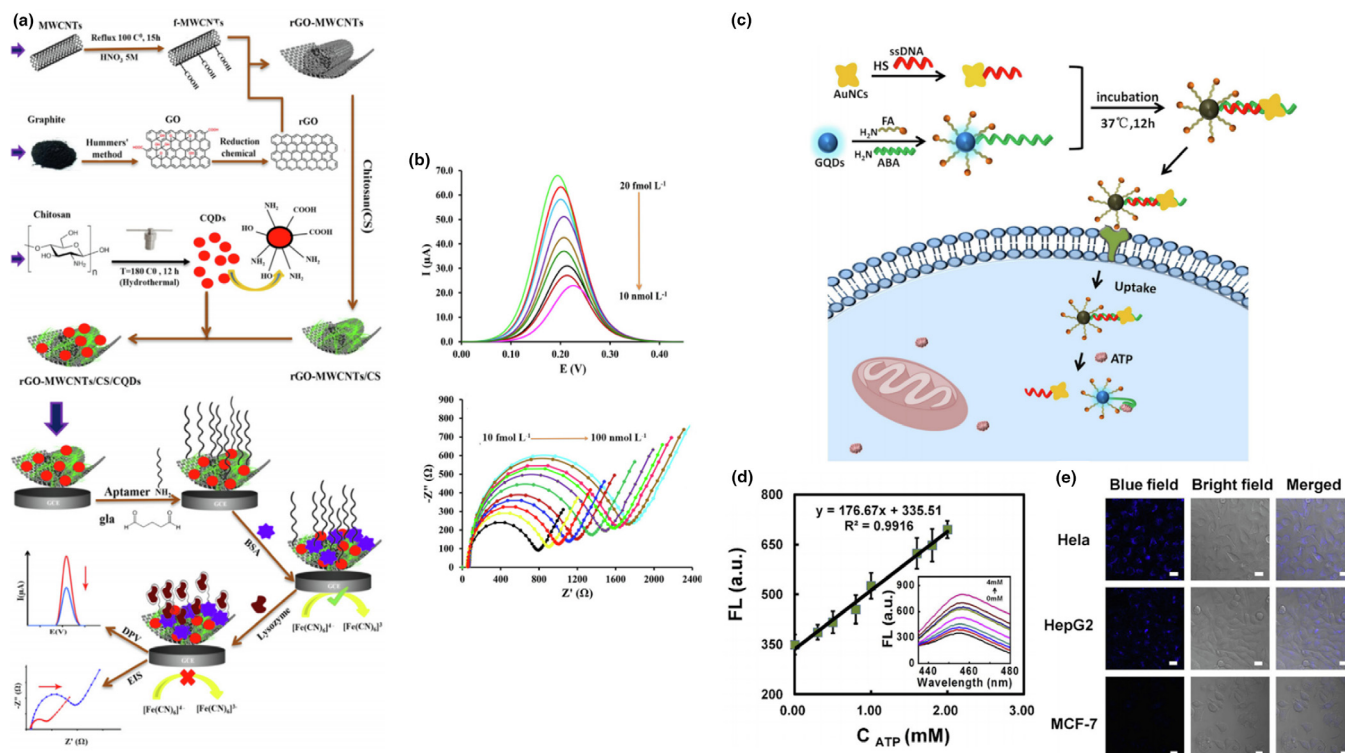


FIGURE 5

An electrochemical aptasensor for the detection of lysozyme developed by Rezaei et al. (a) and (b). In their system (a), a glassy carbon electrode modified with multiwalled carbon nanotubes, reduced GO, chitosan, and CQDs was used as the base substrate. This system was modified with an aptamer specific to lysozyme, and then blocked with bovine serum albumin to prevent non-specific binding. This biotransducer, in the absence of the target analyte, was electrochemically competent and could facilitate the redox reaction of ferri-/ferrocyanide. Upon a binding event, the electrode was passivated, which could be monitored electrochemically via the redox current or electrically with EIS. (a) Highlights the amperometric and impedimetric response of the system. A photoluminescent, intracellular aptasensor developed by Zhang et al. for the detection of ATP (c)–(e). In their system, detailed in (c), they developed a probe which consisted of the quenching pair gold nanocrosses and GQDs. The nanocrosses were conjugated to a strand of ssDNA, and the GQDs were conjugated to folic acid for tumor targeting and to the ATP aptamer. In the presence of ATP, the aptamer would preferentially bind to ATP, dissociating the complex, and restoring fluorescent capacity of the GQDs. (d) Shows the resulting standard curve in the presence of ATP, respectively. (e) Shows the intracellular sensing capacity of the system in various cell lines. (a) and (b) Reproduced from Ref. [193], © 2018 Elsevier. (c), (d), and (e) reproduced from Ref. [187], © 2019 Elsevier.

duced to open the hairpins and probe for the miRNA target of interest. After hybridization, an enzyme which specifically degrades RNA/DNA hybrids was introduced to degrade the hybridized complexes and expose a short leftover sequence from the original hairpin DNA. Repeating these steps forms an array-type of assay. After cycling was completed, three new short DNA oligonucleotides were introduced which were expected to complex with the short, exposed DNA strands, leftovers from the original hairpin DNA strand, and subsequently crosslink forming a large web-like complex. Doping this complex with Ag nanoparticles enhanced the ECL signal. Their system boasted an excellent LOD of 0.83 fM and had translatable potential to allow for measurements of RNA expression levels within cells; their biosensor lined up well with a ubiquitous biological technique, real-time quantitative polymerase chain reaction (RTqPCR).

Recently, Liu et al. described a system for miRNA detection which used a nucleic acid nicking enzyme (Nb.BbvCI) that hydrolyzes only one strand of the duplex to provide signal amplification for ECL readout [197]. The assay involved conjugation of CQDs to an initial DNA sequence, which would hybridize with the miRNA target and an assistant probe respectively. This

complex was recognized by the nicking enzyme, which would then cut the DNA complex and release the miRNA for another hybridization event. The newly cut intermediate DNA strand could then hybridize with a detection hairpin immobilized on a GO electrode to lead to an ECL readout. Their system had a very wide range from 10 aM to 100 nM, with the LOD also being 10 aM. Very recently, Jie et al. reported an ECL sensing system for DNA sequences which relied on the ECL quenching capabilities of Au nanoparticles on GQDs [198]. A GQD solid-state electrode was first fabricated and quenched with Au nanoparticles conjugated to a specific DNA hairpin. A novel aspect of this sensor was the involvement of a DNA polymerase which served as the main signal amplification propagator. When the target DNA was bound to a template DNA complex in solution, the polymerase would bind and fill the distance between the target DNA and the template. This newly synthesized DNA strand would then be nicked by the first endonuclease. This newly released DNA strand would subsequently hybridize with the original hairpin DNA strand bound to the Au nanoparticles. A second endonuclease would then cleave the newly hybridized complex, releasing the quenching nanoparticles from the surface of the electrode, restoring the ECL activity of the GQDs.

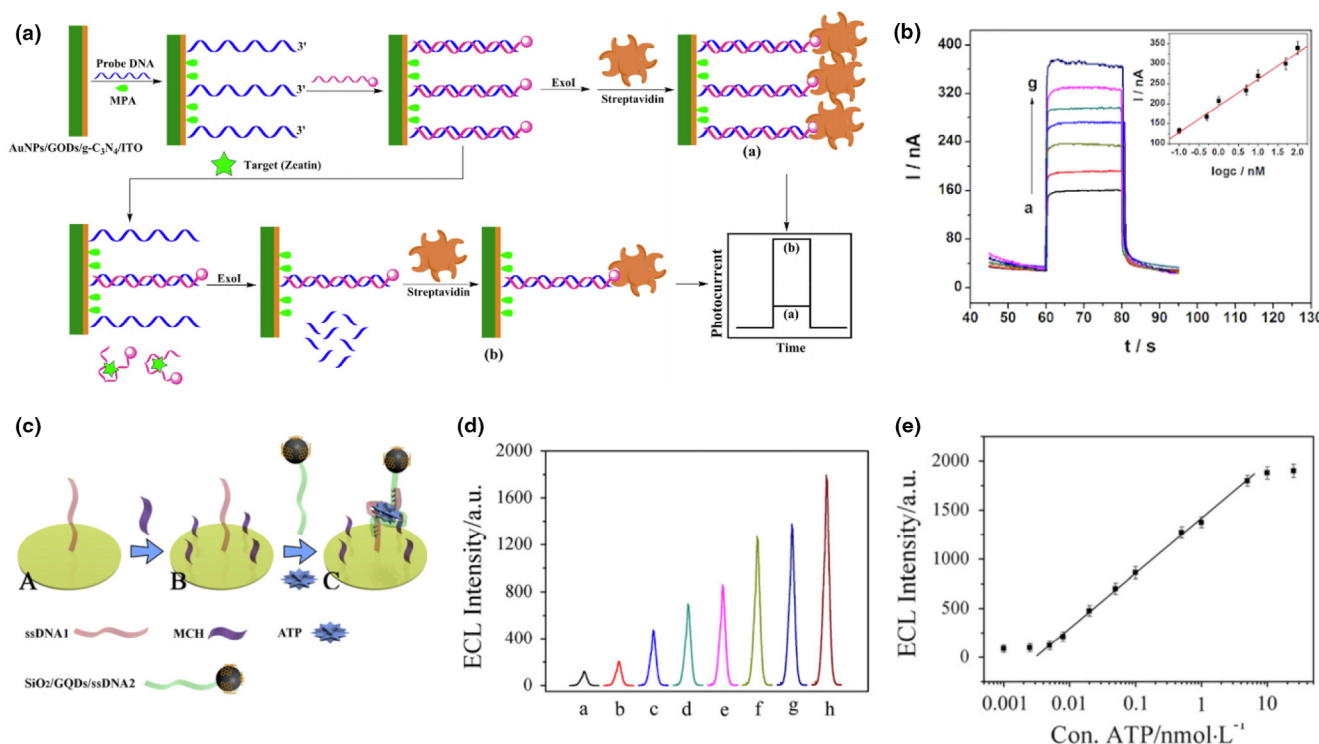


FIGURE 6

A photoelectrochemical (PEC) aptasensor developed by Wang et al. for zeatin detection. In their schematic, the PEC active material was a composite graphite-like carbon nitride doped with GQDs (a) and (b). Detailed in (a), this material was modified with ssDNA responsive to the probe aptameric DNA. In the absence of zeatin, the aptameric sequence would hybridize with the ssDNA on the surface of the electrode, preventing degradation from an ssDNA exonuclease. The aptamer probe was conjugated to biotin, which would hybridize with streptavidin and block PEC current. In the presence of zeatin, the aptamer would preferentially bind to the analyte, and hybridization would not occur. This left the surface-confined ssDNA susceptible to exonuclease activity, the electrode then became unblocked and PEC current spiked. (b) Is the response of the electrode to zeatin, showing both the PEC current and the standard curve. Lu et al. reported an electrochemiluminescent system to detect ATP. Their system (c) was a simple hybridization schematic wherein an electrode was modified with one ssDNA strand and the complementary strand was conjugated to an SiO₂/GQD particle, which served as the ECL source. In the presence of ATP, the two would hybridize, and the ECL intensity would increase. (d) and (e) Show, respectively, the ECL response to increasing concentrations of ATP and the linear response range after a logarithmic transform. (a) and (b) Reproduced from Ref. [201], © 2018 Elsevier. (c), (d), and (e) Reproduced from Ref. [81], © 2013 Elsevier.

Photoelectrochemical biosensors

A photoelectrochemical biosensor using GQDs was developed by Liu et al. and used to detect the antibiotic, chloramphenicol [199]. The authors modified an electrode with nitrogen-doped GQDs and conjugated a chloramphenicol aptamer to the GQDs via π -interactions. When a binding event occurred, an interaction between the chloramphenicol and photoexcited GQDs took place, enhancing the observed photocurrent. Jiang et al. reported a similar system where the photocurrent was shut off upon aptamer interaction with an insecticide, acetamiprid [200]. GQDs were immobilized onto MoS₂ nanosheets, forming an electrical connection where charge transfer occurred upon photonic stimuli. Aptamer binding impeded this transfer, decreasing the photocurrent with higher concentrations of analyte. Wang et al. described a DNA/exonuclease assay-based biosensor for the detection of the plant growth hormone, zeatin (Fig. 6a-b) [201]. Cheng et al. reported a turn-off CQD aptasensor for thrombin, using a similar principle as that used by Jiang et al. [202]. Upon thrombin binding, the photocurrent decreased and a read-out was then tractable. You et al. reported recently an aptasensor for detection of sulfadimethoxine that relied on a charge transfer

cascade between Bi₂S₃ and GQDs [203]. The staggered cascade increased photocurrent on aptamer complexation.

Summary

There are clear benefits to aptasensing as the exceptional specificity of these nucleic acid systems confers excellent performance across many different physiochemical transducers. One of the key prospects of aptasensing in general with GQDs and CQDs is real-time biological monitoring of gene expression. This has exceptional power for *in vivo* and *in vitro* biosensing, as real-time sensing of RNA strands within a cell provides an avenue to detect expression levels of genes without having to fix and isolate cells and perform conventional methods (e.g. PCR). There are some drawbacks across these systems however. Fluorescent sensors may have issues as manifested by the general non-specific binding pattern of nucleic acids with GQDs/CQDs. Additionally, electrochemical endeavors generally require supplement as aptamers rarely are innately conductive – though impedance-based studies will improve this avenue provided non-specific interactions are limited. And lastly, while clearly PEC and ECL methodologies report vast sensing capacity, these

systems are generally sophisticated with some scale-up and application concerns for stability and reproducibility. Nonetheless, a unique benefit is that aptameric sensing allows for nucleic acid signal amplification. There is also great opportunity to improve biological endeavor. One could foresee aptameric probes design to be downstream detectors of molecular pathways – facilitating biological mechanism studies which provide a real-time fluorescent readout for activation of a specific molecular pathway in a dose-responsive manner. To complement the RNA expression analysis, paired systems of aptameric probes can also provide differential monitoring of protein and RNA expression levels and allow for precise measurement of how each of these cellular processes are monitored – allowing for orthogonal, complementary data to conventional proteomic methods. Streamlined assays may even facilitate next-gen sequencing as mediated by GQDs and CQDs. See Table A2 for a technical summary.

Immunobiosensors

Antibodies are large (~150 kDa, hydrodynamic radius, $R_H = 5.25$ nm) proteins generated by the immune system to recognize foreign epitopes and antigens and protect against infection and disease. Antibodies are employed extensively in molecular biology, immunosensing, and clinical medicine. One of the benefits of antibodies is their double-armed structure, which facilitates binding to more than a single target, and in

addition they may bind to each other and be conjugated with enzymes. Complexed systems of antibodies can then comprise unique methods for signal amplification and multiplexed sensing. Despite several excellent attributes as biorecognition elements, there are important drawbacks. Antibodies have varying affinities for the target analyte, with K_D ranging from micromolar (10^{-6}) to picomolar (10^{-12}). Additionally, antibodies have a breadth of analyte specificity, or goodness-of-fit, to the target epitope depending on clonality, and unfortunately off target effects can drastically hinder their capacity for sensing specificity in complex biological media. Polyclonal antibodies (from a B-cell population) have a higher propensity for off-target binding; conversely, for monoclonal antibodies (a single B-cell), this effect is rare. This is commonly quantified via Western blot with observation of a single band on total protein isolates, though more rigorous methods using mass spectrometry may be used should the conventional methods not be conclusive. There is also a size limitation pertaining to raising antibodies to specific small molecule antigens; small molecules are not innately conducive to raising an immune response, though they may be able to bind to the antibody. This could raise obstacles to generating antibodies for the specific target. Conjugation of the analyte to a larger construct may be required, generating a hapten. Finally, the binding properties of an antibody are highly dependent upon environmental factors such as ionic strength, and often differ from one

TABLE A2

Aptamer, peptide, and genomic-based biosensing at a glance.

Authors	Biorecognition	Analyte	Linear range	Limit of detection	Notes	Refs.
<i>Photoluminescent and colorimetric biosensors</i>						
Loo et al.	ssDNA	DNA	0.4–400 nM	17.4 nM	General System	[178]
Shi et al.	ssDNA	<i>S. aureus</i> mecA	–	1 nM	Gene Detection	[179]
Liang et al.	dsDNA	dsDNA	0–50 nM	1 nM	On/Off Sensor	[180]
Wang et al.	Hairpin Loop (DNA)	p53 DNA Mutants	1 pM–1.5 nM; 1.5–50 nM	0.8 pM	Gene Detection	[181]
Kermani et al.	dsDNA	M.SssI	5–30 U/mL	0.7 U/mL	Enzyme Activity	[182]
Wang et al.	DNA Aptamer	Thrombin	0.5–20 nM	180 pM	On/Off Sensor	[183]
Zhu et al.	DNA Aptamer	Dopamine	0.1–5 nM	55 pM	On/Off Sensor	[184]
Shi et al.	DNA Aptamer	EpCAM	3–54 nM	450 pM	On/Off Sensor	[185]
Lou et al.	DNA Aptamer	ATP	0–900 nM	3.3 nM	Strand Displacement	[186]
Zhang et al.	DNA Aptamer	ATP	0–2 mM	0.27 mM	Intracellular	[187]
Li et al.	Peptide	Trypsin	0–1 μ M; 10–400 μ M	33 ng/mL	On/Off Sensor	[188]
Su et al.	Peptide	Trypsin	0.03–8 μ g/mL	6.3 ng/mL	On/Off Sensor	[189]
Wang et al.	Peptide	Casein Kinase II	0.1–1.0 U/mL	0.025 U/mL	Enzyme Activity	[190]
<i>Electrochemical biosensors</i>						
Zhao et al.	DNA Aptamer	Thrombin	200–500 nM	100 nM	Amperometric	[191]
Peng et al.	dsDNA	Horseradish Peroxidase	1–40 U/mL	0.3 U/mL	Amperometric	[192]
Rezaei et al.	DNA Aptamer	Lysozyme	10 fM–100 nM	1.9 fM	Impedimetric	[193]
Huang et al.	DNA Aptamer	Carcinoembryonic Antigen	0.5 fg/mL–0.5 ng/mL	0.34 fg/mL	Amperometric	[194]
Hu et al.	ssDNA	miRNA-155	1 fM–100 pM	0.14 fM	Amperometric	[195]
<i>Electrochemiluminescent biosensors</i>						
Lu et al.	ssDNA	ATP	5 pM–5 nM	1.5 pM	ECL Turn On	[81]
Zhang et al.	Hairpin Loop (DNA)	miRNA	2.5 fM–50 pM	0.83 fM	Exonuclease Amplification	[196]
Liu et al.	ssDNA	miRNA	10 aM–100 nM	10 aM	ECL Turn On	[197]
Jie et al.	ssDNA	ssDNA	1 pM–1 μ M	0.1 pM	Polymerase Amplification	[198]
<i>Photoelectrochemical biosensors</i>						
Liu et al.	DNA Aptamer	Chloramphenicol	10–250 nM	3.1 nM	PEC Turn On	[199]
Jiang et al.	DNA Aptamer	Acetamidiprid	0.05 fM–1 nM	16.7 fM	PEC Turn Off	[200]
Wang et al.	DNA Aptamer	Zeatin	0.1–100 nM	0.031 nM	PEC Turn On	[201]
Cheng et al.	DNA Aptamer	Thrombin	1–250 pM	0.83 pM	PEC Turn Off	[202]
You et al.	DNA Aptamer	Sulfadimethoxine	0.1–120 nM	0.03 nM	Z-Scheme Charge Transfer	[203]

antibody to another, making optimization for a multiplex system difficult. Despite this, the benefits and binding propensities of antibodies are overall excellent and have great prospects in biosensing.

Photoluminescent and colorimetric biosensors

Zhao et al. described a generally translatable system using antibodies (Abs) conjugated to GQDs for antigen detection (Fig. 7a–b) [204]. The Ab/GQD conjugate was adsorbed onto graphene, which quenched the fluorescence of the GQDs; upon binding of the Ab to the antigen (Ab-Ag), the GQDs were liberated and fluorescence restored. In their proof-of-concept system with IgG, they were able to detect a specific antigen down to a concentration of 10 ng/mL. Using this scheme, Bhatnagar et al. reported a fluorescence biosensor comprised of GQDs chemically conjugated to anti-cardiac troponin I Ab for detection of a heart attacks [205]. Thanks to the excellent performance of Abs for analyte detection, authors reported a wide linear range of detection from 1 pg/mL to 1 µg/mL and an LOD of 192 fg/mL.

A particularly novel immunosensing platform that used the peroxidase-mimicking activity of Ir-Pd nanocubes and CQDs as the reference fluorophore as a readout was recently reported by Tan et al. [206]. In their report, they described a sandwich

immunosensor for detecting cardiac troponin I; upon complexation, the nanocubes would catalyze the reaction and oxidize o-phenylenediamine to a fluorescent product, which in turn would quench the CQDs and yield a ratiometric sensor. Their system, notably, had good performance in serum and additionally compared favorably to enzyme-linked immunosorbent assays.

Electrochemical biosensors

Earlier work incorporating GQDs into electrochemical immunosensors exploited the high surface area to volume ratio offered by GQDs, as reported by Wang et al. [207]. In their work, GQDs were used as scaffolds to hold one of the two Abs used in the system and thus to serve as carriers for a signal amplifier (Cu^{2+} /apoferritin). The other Ab was conjugated directly to the electrode, and in the presence of the antigen, both Abs would complex (sandwich design) and a signal readout was attained from the electroactive cargo on the GQD. For viral detection, their system had good performance and was able to measure tissue infection values well into pathophysiologically relevant ranges. Tuteja et al. used the electrical properties of GQDs to monitor changes in charge transfer resistance (R_{CT}) as antigens bound to immunosensor [208]. They fabricated a GQD-modified electrode and conjugated a myoglobin Ab to its surface.

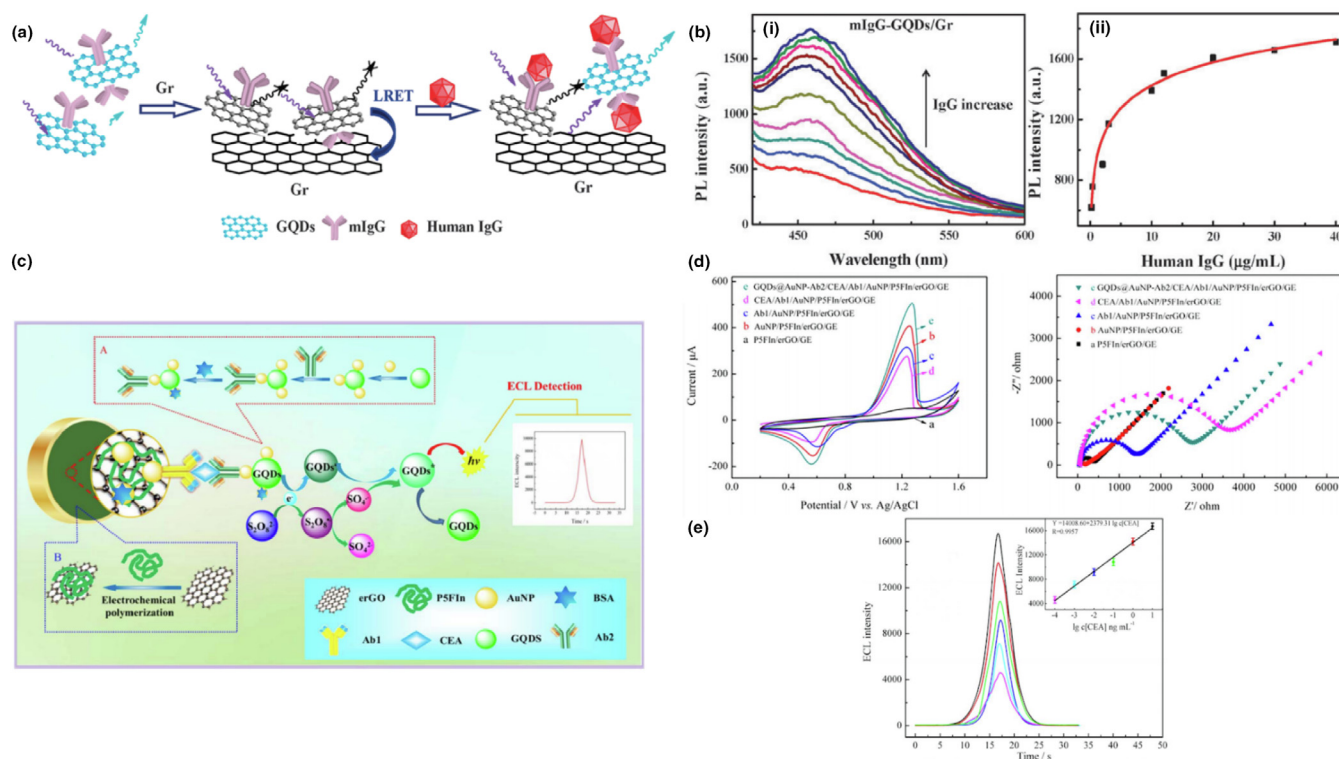


FIGURE 7

A universal photoluminescent immunosensing regime using graphene and GQDs detailed by Zhao et al. (a) and (b). In (a), GQDs modified with a target-specific antibody (mouse anti-human IgG in this work) were free to interact with graphene in the absence of the analyte, resulting in the quenching of the GQDs. In the presence of the target epitope, the interaction between the GQDs and the graphene would be sterically inhibited, and the GQDs would again begin fluorescing. (b) Shows the increasing fluorescent intensity as a function of target concentration (human IgG in this work; left), and the dose response curve of intensity vs. analyte concentration (right). An electrochemiluminescent (ECL) immunosensor from work by Nie et al. with an application to cancer biosensing (c)–(e). They designed a sandwich immunosensor for the detection of carcinoembryonic antigen (CEA, c), where the dual sandwich binding event would result in an appreciable increase in ECL intensity. (d) Shows the characteristic cyclic voltammograms (CVs) of their system during stepwise synthesis and antigen presence (left) and their respective EIS responses. (e) Shows the increase in ECL intensity as the concentration of CEA increases and the resulting standard curve. (a) and (b) Reproduced from Ref. [204], © 2013 Royal Society of Chemistry. (c), (d), and (e) Reproduced from Ref. [217], © 2018 Elsevier.

Following myoglobin binding, interfacial charge transfer was compromised and R_{CT} was increased. For myoglobin, authors reported a linear range from 0.01 to 100 ng/mL and an LOD of 10 pg/mL, which they noted was comparable to ELISA. A screen printed sensor was developed by Mehta et al. for parathion sensing [209]. In their system, they first functionalized an electrode with GQDs followed by a parathion antibody and monitored changes in R_{CT} , with increased resistances correlating positively with higher analyte concentrations. Yang et al. developed a unique immunosensor that used the electrocatalytic properties of GQDs for CEA sensing [210]. They reported a surface modified electrode of platinum-palladium (PtPd) nanoparticles, GQDs, and Au nanoparticles further conjugated to a CEA antibody. Increasing concentrations of CEA led to increased binding at the surface of the electrode which sterically limited access by H_2O_2 and subsequent amperometric decrease. Malekzad et al. reported a system that used an Ab to detect prostate-specific antigen that was observed electrochemically as a new oxidative peak on a GQD-modified electrodes doped with the Ab; with the current increasing with the Ab concentration [211]. Mollarasouli et al. used a similar scheme to detect receptor tyrosine kinase, although they monitored the current of a redox indicator reaction (ferri-/ferrocyanide) at the GQD-modified electrode as antigen bound, passivating the electrode and reducing the observed current [212]. A more elaborate biosensor was reported

recently by Serafin et al. which they employed for IL-13 detection (Fig. 8) [213]. Each of these studies reported strong LODs reaching down into the ng or pg range.

Electrochemiluminescent biosensors

An ECL sandwich immunosensor system was reported by Yang et al. wherein one of the Abs was directly conjugated to GQDs. In the presence of the antigen, the GQDs/antibody complex would bind and the ECL intensity would increase [214]. A similar assay was designed by Zhou et al. as an ECL biosensor for the detection of α -fetoprotein [215]. In their system, the modified CGD electrode would exhibit ECL that was quenched when Ab-modified graphene was present and sandwich binding took place. A similar system for α -fetoprotein detection was reported by Zhang et al. [216] and recently for carcinoembryonic antigen by Nie et al. (Fig. 7c–e) [217]. Each of these systems used a sandwich conjugation scheme and GQD ECL increase as the readout. An ECL passivation assay was reported by Zhang et al. wherein they recorded ECL intensity from a CQD-surface modified electrode for the detection of 8-hydroxy-2'-deoxyguanosine [218]. Upon binding of the antigen to the Ab/CQD electrode, electron transfer was inhibited and ECL decreased. A similar passivation schematic was employed by Wu et al. to detect prostate specific antigen [219] and by Dong et al. to detect carcinoembryonic antigen [220].

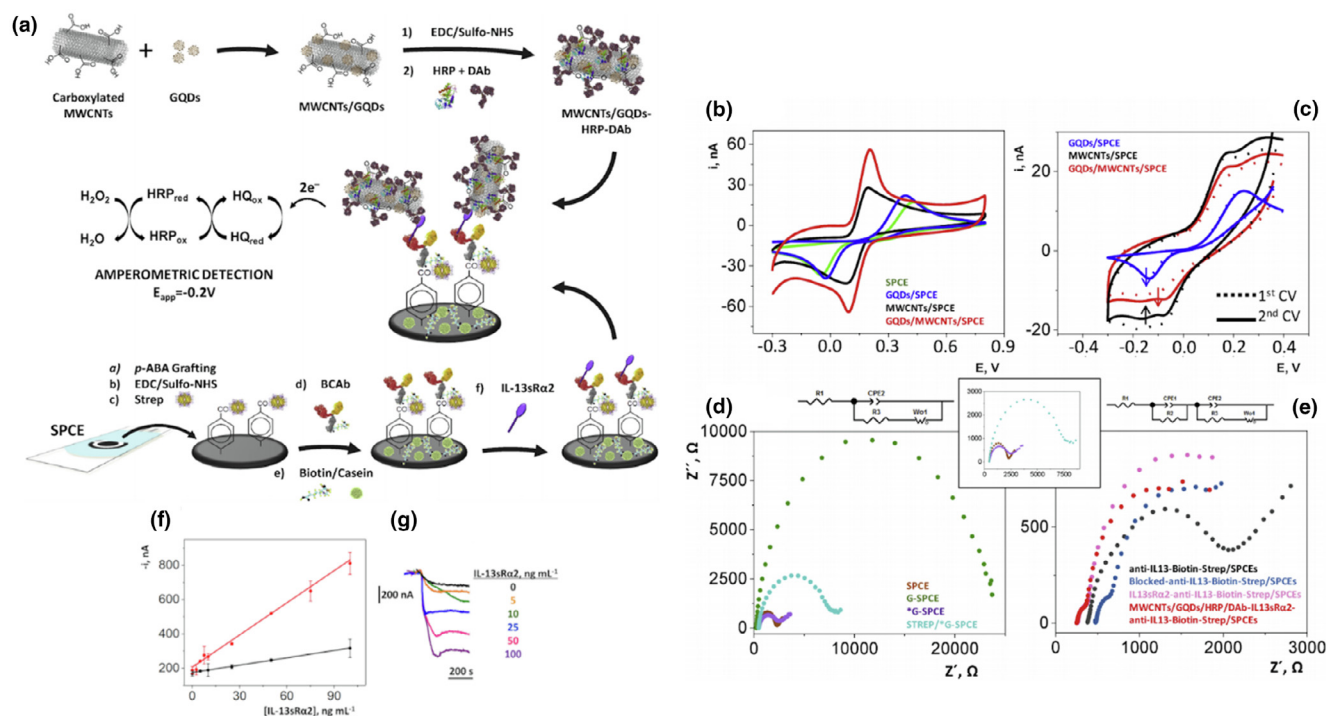


FIGURE 8

(a) Details an electrochemical sandwich immunosensor developed by Serafin et al. for the detection of IL-13 receptor α 2. One half of the sandwich was a GQD-modified CNT complex doped with HRP and a detector antibody. The other half was a carbon electrode modified with streptavidin/para-aminobenzoic acid, which in turn immobilized the capture antibody conjugated to biotin. On a dual binding event, the HRP was brought in close proximity to the electrode, and the H_2O_2 byproduct could be detected amperometrically. Various CVs of the different surface modified electrodes in the presence of the precursor (hydroquinone, b), and the precursor plus 100 mM H_2O_2 (c). (d) Is the electrochemical impedance spectroscopy (EIS) response of the system during the various surface modification steps, while (e) shows the EIS of the full system in the presence and absence of the analyte. Equivalent circuits that were used to fit the data are detailed above each of their respective EIS spectra (d) and (e). Reproduced from Ref. [213], © 2019 Elsevier.

Photoelectrochemical biosensors

Tian et al. built a photoelectrochemical immunosensor to detect microcystin-LR [221]. In their system, authors coated silicon nanowires with GQDs to enhance the overall photocurrent, then doped the system with an Ab specific to their target. Antigen blocked the photocurrent within the system, resulting a decrease in current with increasing antigen concentrations. A unique photoelectrochemical approach was devised by Lin et al. which involved enzymatic dissolution of an oxide nanosheet supporting CQDs [222]. In their system, the authors labeled the analyte of interest with GOx and pre-spiked the detection medium; a binding event would localize H₂O₂ production to the nanosheet, etching it and releasing the CQDs, ultimately resulting in photocurrent decreasing. Addition of the native analyte outcompeted the conjugate, protecting the sheet from etching and preserving ECL. A similar quenching style system was reported by Lv et al. using a sandwich approach [223]. In their system, the CQD/g-C₃N₄ complex provided an initial photocurrent, and upon sandwich immunocomplex formation, Cu²⁺ ions loaded into nanospheres were released and photocurrent was quenched. Interestingly, the authors loaded the nanospheres onto an aptamer, which would release the Cu²⁺ ions upon antibody binding. An acid/base chemistry approach was reported by Gong et al. [224]. In their system, oxidized CQD/TiO₂ nanoparticles had their initial strong photoelectrochemical capacity shut off. They performed the immunoassay in microwell plates, with alkaline phosphatase catalyzing the formation of ascorbic acid,

which would subsequently reduce the CQD complex and restore the photocurrent.

Summary

There are various avenues which may be further explored by antibody functionalized GQDs and CQDs. Orthogonally functionalized systems facilitate a highly specific ratiometric sensing regime, rather than a single readout. Another interesting avenue to explore for intracellular sensing is FRET-based GQD sensing, where differentially functionalized GQDs or CQDs that form a FRET pair may be used to investigate and sense biological interactions. Additional extension to solid state, wearable sensors or microfluidic systems is facilitated strongly by immunosensing as well. For example, a single, linear channel with discrete islands of antibody functionalized electrodes can serve for multi-level sensing in complex biological fluids without a need to modify each sensing space beyond the antibody functionalization. This is also an ideal avenue for wearable sensors, as such a system is not too far-removed from the ones described here. See Table A3 for a technical summary.

Biomolecular and supramolecular biosensing

This section gives a full treatment to biosensing regimes which do not fall cleanly in the previous sections. Incidentally, by the strict definition of supramolecular chemistry, every biotransducer in this review is a supramolecular sensor as it consists of interfacial chemistries between compounds. This section, thus,

TABLE A3

Immunobiosensing at a glance.

Authors	Biorecognition	Analyte	Linear range	Limit of detection	Notes	Refs.
<i>Photoluminescent and colorimetric biosensors</i>						
Zhao et al.	Mouse IgG	Human IgG	0.2–12 µg/mL	10 ng/mL	Universal System	[204]
Bhatnagar et al.	Antibody	Cardiac Troponin I	1 pg/mL–1 µg/mL	192 fg/mL	On/Off Sensor	[205]
Tan et al.	Antibody	Cardiac Troponin I	1 pg/mL–1 ng/mL	0.31 pg/mL	Sandwich, On/Off Sensor	[206]
<i>Electrochemical biosensors</i>						
Wang et al.	Antibody	Avian Leukosis Virus J	10 ^{2.08} –10 ^{4.50} TCID ₅₀ /mL	115 TCID ₅₀ /mL	Sandwich, Amperometric	[207]
Tuteja et al.	Antibody	Myoglobin	0.01–100 ng/mL	10 pg/mL	Impedimetric	[208]
Mehta et al.	Antibody	Parathion	0.01 ng/L–1 mg/L	46 pg/L	Impedimetric	[209]
Yang et al.	Antibody	Carcinoembryonic Antigen	5 fg/mL–50 ng/mL	2 fg/mL	Amperometric	[210]
Malekzad	Antibody	Prostate Specific Antigen	2–9 pg/mL	1.8 pg/mL	Amperometric	[211]
Mollarasouli et al.	Antibody	Tyrosine Kinase	1.7–1000 pg/mL	0.5 pg/mL	Amperometric	[212]
Serafin et al.	Antibody	IL-13	2.7–100 ng/mL	0.8 ng/mL	Amperometric	[213]
<i>Electrochemiluminescent biosensors</i>						
Yang et al.	Antibody	L-Cysteine	0.002–70 U/mL	0.96 mU/mL	Sandwich, ECL Turn On	[214]
Zhou et al.	Antibody	α-Fetoprotein	0.01–100 ng/mL	3.3 pg/mL	Sandwich, ECL Turn Off	[215]
Zhang et al.	Antibody	α-Fetoprotein	0.005–100 ng/mL	1.2 pg/mL	Paper, Sandwich, ECL Turn On	[216]
Nie et al.	Antibody	Carcinoembryonic Antigen	0.1 pg/mL–10 ng/mL	3.78 pg/mL	Sandwich, ECL Turn On	[217]
Zhang et al.	Antibody	8-Oxo-2'-deoxyguanosine	0–200 ng/mL	85 pg/mL	ECL Turn Off	[218]
Wu et al.	Antibody	Prostate Specific Antigen	1 pg/mL–10 ng/mL	0.29 pg/mL	ECL Turn Off	[219]
Dong et al.	Antibody	Carcinoembryonic Antigen	0.02–80 ng/mL	0.01 ng/mL	ECL Turn Off	[220]
<i>Photoelectrochemical biosensors</i>						
Tian et al.	Antibody	Microcystin-LR	0.1–10 µg/mL	55 ng/mL	PEC Turn Off	[221]
Lin et al.	Antibody	Aflatoxin	0.01–20 ng/mL	2.1 pg/mL	Competitive PEC Turn On	[222]
Lv et al.	Antibody	Prostate Specific Antigen	0.02–100 ng/mL	5 pg/mL	PEC Turn Off	[223]
Gong et al.	Antibody	Carcinoembryonic Antigen	0.01 pg/mL–30 ng/mL	7 fg/mL	PEC Turn On	[224]

serves to discuss biotransducers that do not use the common biorecognition elements (enzymes, nucleic acids, and antibodies). Doing so explores unique chemistries and interfaces. Examples of such supramolecular biosensors would be the use of porphyrin molecules, protein or genomic motifs (such as a zinc-finger, or the G-quadruplex in DNA), or large organic molecules. This research division is considerably smaller in size compared to other sections, but represents an enormous opportunity for innovation and exploration.

Photoluminescent and colorimetric biosensors

One of the more extensively studied interactions of GQDs and CQDs is with porphyrinic compounds, organic heterocyclic macrocycles composed of four modified pyrrole subunits interconnected at their α carbon atoms via methine bridges ($=CH-$) [135,136,225,226]. An early study developed a biosensor based on the quenching behavior of hemin and GQDs [227]. He et al. described a system where hemin was complexed with GQDs via $\pi-\pi$ stacking. This compound would be quenched in the presence of H_2O_2 , which they coupled to GOx for a glucose biosensor. Authors attributed the quenching behavior to the production of free radicals, which destroyed the surface states of the GQD and subsequently shut off fluorescence. Zhang et al. built a turn-on biosensor using a different porphyrin molecule [228]. In the presence of H_2O_2 , the metalloporphyrin would rupture and lose the Fe^{III} metal ion and its quenching capacity. Two groups reported using dopamine functionalized GQDs/CQDs as fluorescent biosensors [229,230]. These systems employed dopamine-conjugated particles as a probe to detect Fe^{III} (Chowdhury et al.) or tyrosinase activity (Chai et al.); in both systems, the dopamine was a reactive biomolecule where upon reaction with the desired analyte would turn off fluorescence. A unique approach using GQDs was reported by Huang et al., where they used a G-quadruplex/hemin supramolecular

sensor to detect human telomeric DNA (Fig. 9) [231]. Their sensor boasted a linear range of 0.2–50 pM and an LOD of 25 fM.

Electrochemical (EC) biosensors

Electrochemical supramolecular biosensors that do not employ redox enzymes are still relatively underexplored. Shadjou et al. used a large biomacromolecular compound, β -cyclodextrin, to serve as a chemical “net” for L-tyrosine detection [232]. Authors electrodeposited GQDs conjugated to β -cyclodextrin onto glassy carbon electrodes and monitored changes in oxidative current upon binding events. They isolated the effect of the β -cyclodextrin, which provided a slight current amplification from the current of a simple GQD modified electrode. They reported a limit of quantification of 100 nM.

Summary

Supramolecular biosensing is a broad field with few studies falling into this section based on the relative scarcity of use of biomolecular motifs. Thus, forays into these systems will be novel based on the unique molecular recognition element incorporated. Some unexplored examples include protein/metal motifs and demetalized constructs thereof, providing a structurally mediated sensor for metal cofactors in biological systems, as metal ions tend to interact optically with GQDs and CQDs. Isolated ion channel selectivity filters are one such tangible example. Additionally, many metallized supramolecular complexes in biology have pertinent electrochemical activity (e.g., iron prosthetic groups) and may facilitate electrochemical, ECL, and PEC sensor development, all of which are currently scarce or absent from the current literature. See Table A4 for a technical summary.

Conclusions and future directions

Conclusions and summary

In this review, recent advances were discussed involving the development of biotransducers and biosensor systems which

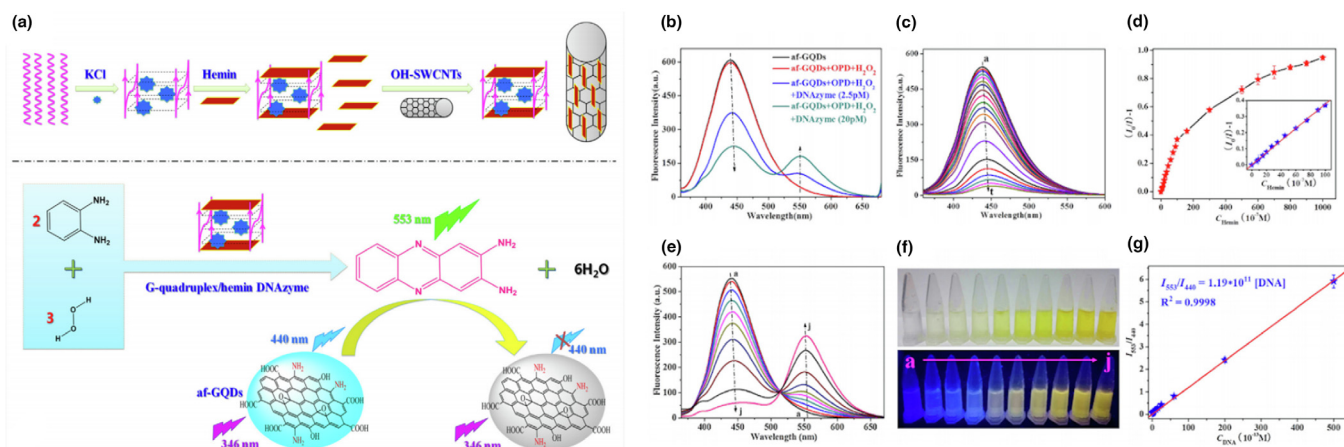


FIGURE 9

A supramolecular biosensing regime developed by Huang et al. for the quantification of human telomeric DNA. They coupled the DNAzyme activity of the hemin/G-quadruplex complex (which forms in human telomeric DNA sequences) with the photoluminescent quenching of GQDs. Pictured in (a) is a rendition of a parallel quadruplex structure, though other configurations are possible. The DNAzyme was capable of converting ortho-phenylenediamine to 2,3-diaminophenazine, which in turn quenched GQDs and formed a turn-off biosensor (a). (b) Shows the fluorescent spectra of varying compounds and system configurations, and (c) shows the fluorescent quenching of GQDs in the presence of hemin (plotted as a standard curve in d). (e) Shows the ratiometric response of the GQD system to varying concentrations of hemin/G-quadruplex DNAzyme, with the associated colorimetric response shown in (f). This response was visualized as a standard curve shown in (g). Reproduced from Ref. [231], © 2017 Elsevier.

TABLE A4

Biomolecular and supramolecular biosensing at a glance.

Authors	Biorecognition	Analyte	Linear range	Limit of detection	Notes	Refs.
<i>Photoluminescent and colorimetric biosensors</i>						
He et al.	Glucose Oxidase/Hemin	Glucose	9–300 μ M	0.1 μ M	On/Off Sensor	[227]
Zhang et al.	Metalloporphyrin	H ₂ O ₂	2–300 μ M	0.3 μ M	On/Off Sensor	[228]
Chowdhury et al.	Dopamine	Fe ^{III}	20 nM–2 μ M	7.6 nM	On/Off Sensor	[229]
Chai et al.	Dopamine	Tyrosinase	0–800 U/L	7 U/L	Intracellular, Enzyme Activity	[230]
Huang et al.	G-Quadruplex/Hemin	Telomeric DNA	0.2–50 pM	25 fM	Ratiometric	[231]
<i>Electrochemical biosensors</i>						
Shadjou et al.	β -Cyclodextrin	L-tyrosine	0.1–1.5 μ M	100 nM	Amperometric	[232]

were enabled by either GQDs or CQDs, with most progress occurring in the past 5 years. One reason for the fledgling nature of the literature is the relatively cryptic properties of GQDs and CQDs and engineering the interface requires a degree of empiricism. Nevertheless, there has been considerable headway made in developing biosensors that use the properties of GQDs and CQDs. Studies on the electron transfer kinetics for enzymatic DET have been fruitful, as much of the difficulty of interfacing the above nanomaterials with the redox active centers within enzymes is remedied through GQD/CQD electrochemical biosensors. This captures the power of the engineered biointerface because GQDs and CQDs have length scales compatible with redox active sites i.e. within tunneling distance of cofactors, they combine excellent electron transfer and electronic conduction capabilities. Furthermore, they are relatively non-cytotoxic, and their π - π adsorption chemistry offers compatible chemistry to support the enzymes without structural or functional interference. Worth highlighting is also the recent development of PEC and ECL biosensors using these nanodots. This area of research integrates the luminescent properties of GQDs/CQDs with their electronic properties – leveraging their characteristics to set them apart from other nanomaterials.

Prospects and future directions

There are still many places for improvement in GQDs and CQDs with several groups focusing on chemical modification and general applications of the two systems. When consolidating current research conducted on GQDs and CQDs, much of it can be distilled to the simple framework of “biocompatible” semiconductor quantum dots. Indeed, much of their application is generally not lost should they be replaced with their semiconductor counterparts. In many of the recent studies, semiconductor QDs are even incorporated to amplify the luminescent properties of GQDs or CQDs. With this in mind, it is puzzling that exploration into real-time biological sensing is relatively absent from literature. This is an accessible pathway to leverage the most striking benefit that GQD and CQD incorporation allows – nanobeacons for intracellular real-time signaling to provide fundamental insight into cellular processes and kinetics not allowed by many conventional biological techniques. For processes which can be monitored, GQDs and CQDs offer a simple route to detection and monitoring with overall relatively simple designs and effectiveness.

An important perspective is that much of the surface chemistries and electronic states which govern the properties of GQDs

and CQDs are still nebulous and thus challenging to control, which leaves surface modification and interfacial engineering somewhat of an empirical process. Due to the luminescent and electronic properties being a mixture of surface states and quantum confinement (among other contributors), it is still within the realm of possibility to destroy or disrupt the properties which facilitated incorporation into the biosensing system initially. Without this underlying fundamental framework, interface engineering of these systems to optimize charge transfer processes remains a challenging task. Additionally, the performance of these materials *in vitro* and *in vivo* can be relatively unpredictable should an undesirable reactive species be present – notably many systems involve chemistries with reactive oxygen species that are known to be present within physiological environments.

This being said, there are several notable benefits accrued to GQDs and CQDs making them strong candidates for future innovations in biosensor development. They interface exceptionally well with the biological recognition elements used, as evidenced by the outstanding performance shown (Appendix). Indeed, in many reports and works reviewed here, the authors translated their work to complex media including serum, blood samples, and fruit juices (in the case of glucose) – and in such contained systems, performance was maintained. Many works therefore do support translation, and the prospects for clinical use is promising. The technical performance of many of biosensors developed thus far reaches well into known pathophysiological ranges – with pico- and femtomolar concentrations commonly reported and some even sensing attomolar signals. Additionally, so far biocompatibility is stronger than other state-of-the-art nanosensing technologies (nanowires, semiconductor dots, and other graphene variants) – which gives them their niche in relation to these other state-of-the-science approaches. Technical performance for nanosensors is universally shared across many of the current technologies, though GQDs and CQDs position themselves with capacity for *in vivo* monitoring and sensing in a way that these other materials do not. Their greatest asset may be their ability to allow for non-toxic intracellular and intravital sensing and monitoring without considerable complications – although more investigation is undoubtedly still required.

Regardless, the current obstacles will be traversed over time as the chemistry, physics, and engineering progress in characterizing and applying these materials. The potential they offer for biosensing modalities is clear, as they bring luminescent, electrochemical, and combinations of these two properties in a single

material, while additionally being non-toxic and generally safe for translational use. This is an exciting time in the field of biosensing and there is a wide space to be investigated for specific applications to answer questions in biology and improve current bioanalytical technologies.

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