



# Carbon Quantum Dots: Green Nano-biomaterials in the Future of Biosensing

# 14

Barbara Vercelli

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## Abstract

Since their casual discovery in 2004, carbon quantum dots (CDs), fluorescent carbon-based nanomaterials, were highly studied by the research community, by virtue of their particular properties, which enable a wide range of biological applications, going from chemo- and biosensing to fluorescence imaging and drug delivery. They are characterized by excellent fluorescence emission, which can be modulated from the visible to near-infrared (NIR), and their surface is rich in functional groups, which enable their functionalization with a wide range of receptors and bio-functionalities (i.e., enzymes, etc.). They also exhibit extremely low toxicity and excellent biocompatibility, which are useful for real-world biological applications. On the other hand, CDs are also expected to reduce the cost of biosensors by replacing both the noble metal substrates and the well-known quantum dots based on metal chalcogenides. Their synthesis is based on simple and sustainable approaches that could employ cheap and “greener”

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U. P. Azad, P. Chandra (eds.), *Handbook of Nanobioelectrochemistry*,

[https://doi.org/10.1007/978-981-19-9437-1\\_14](https://doi.org/10.1007/978-981-19-9437-1_14)

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starting materials coming from biomass or agro-industrial waste. In this context, the present chapter will focus on CDs as new sustainable nanomaterials for biosensor applications, with particular care to optical and electrochemical biosensors. A short overview of the green synthesis strategies of CDs and their optical properties is also presented.

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**Keywords**

Carbon quantum dot · Green synthesis · Biosensors

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## 1 Introduction

Carbon quantum dots (CQDs) (Lim et al. 2015), also called carbon dots (CDs) (Sun et al. 2006; Baker and Baker 2010), constitute a new class of carbon-based nanomaterials, characterized by diameters less than 10 nm and featured by peculiar fluorescence properties. Xu et al. casually discovered CDs during the electrophoretic purification of single-walled carbon nanotubes (SWCNTs) (Xu et al. 2004), and since then, they have seen a rapid development and were considered a case study in materials science (Zhou et al. 2020b; Zheng et al. 2020; Peng et al. 2017). By comparison with the traditional luminescent nanoparticles, CDs present numerous superior characteristics like high photostability, nontoxicity, and low cost (Dou et al. 2016; Zhao et al. 2011; Zheng et al. 2017; Zhou et al. 2017; Dong et al. 2017; Chen et al. 2018; Feng et al. 2016; Li et al. 2014; Zhang et al. 2018; Gao et al. 2016).

In 2016, Valcárcel's group (Cayuela et al. 2016) classified CDs into three different species depending on their nature, crystalline structure, and quantum confinement. They defined carbon nanodots (CNDs) as the amorphous nanodots that are quasi-spherical and do not present quantum confinement; carbon quantum dots (CQDs) as the nanodots that are spherical and show a crystalline structure with quantum confinement; and graphene quantum dots (GQDs) as the single sheets that are  $\pi$ -conjugated. Literature reports that the formation of the above reported different species of CDs depends on both the synthesis strategies developed for their fabrication and the precursors employed as starting materials. The production of CDs comprises a variety of approaches, which are classified into two main groups: "top-down" and "bottom-up." The "top-down" approaches are based on harsh processes that breakdown large pieces of raw carbon materials into CDs. They include approaches like laser ablation (Li et al. 2011), arc discharge (Xu et al. 2004), electrochemical exfoliation (Tan et al. 2015; Lu et al. 2009), and oxidation acid treatment (Lu et al. 2020; Ray et al. 2009), and employ as precursors materials like carbon soot (Tian et al. 2009), carbon fiber (Peng et al. 2012), activated carbon (Qiao et al. 2009), carbon black (Dong et al. 2012), graphene (Pan et al. 2010), and carbon nanotubes (Xu et al. 2004). On the other side, the "bottom-up" approaches employ small molecules as carbon sources, which are converted into CDs through "decomposition–polymerization–carbonization" processes. The usually employed carbon-source molecules include small organic molecules like citric acid (Peng

et al. 2020b; Li et al. 2020; Schneider et al. 2017), polyols (Liu et al. 2014), and amino acids (Sahiner et al. 2019); synthetic polymers like polyethylene glycol (PEG) (Ji et al. 2020; Li et al. 2015) and polythiophene (Ge et al. 2015); and natural products like carbohydrates (Peng and Travas-Sejdic 2009) and polysaccharides (Zhou et al. 2013). The most commonly employed bottom-up synthetic methods are pyrolysis-solvothermal (Chen et al. 2020; Wang et al. 2010), hydrothermal carbonization (Liu et al. 2012), and microwave/ultrasonication (Zhu et al. 2009). Recently, many research efforts were devoted to the development of more sustainable approaches that are based on the following two strategies: the substitution of the organic compound precursors with materials coming from biomass (like orange juice, honey, betel nut shells, silk, etc.) (Jiang et al. 2020a; Huang et al. 2020; Meng et al. 2019; Mandani et al. 2017; Wu et al. 2013); and the development of new processes that should be both efficient and do not require high expenditures of external energy. Thus, owing to the possibility of green preparation methods together with the abovementioned peculiar properties, CDs are expected to replace the conventional inorganic quantum dots or noble metal nanoclusters in biosensing applications and become promising sustainable luminescent nanoprobe. A typical nanoprobe for biosensing applications has to present the following features: (1) wavelength in the near-infrared (NIR) region because the high energy of ultraviolet rays may damage living organisms and biological tissues easily absorb visible light; (2) it has to exhibit chemical inertness and photoluminescence (PL) emission stability; (3) it should be harmless to organisms. Regarding point 1, it was shown that through suitably designed synthetic approaches (Loi et al. 2017; Kozák et al. 2013), adequate selection of carbon sources (Ge et al. 2015), and doping with heteroatoms (Jiang et al. 2020; Sun et al. 2019; Zhang et al. 2012), it is possible to modulate the PL emission of CDs from the visible to the NIR; for point 2, CDs, compared to organic dyes, present the advantage of not being easily metabolized by cells and remain stable in the body; and for point 3, CDs, unlike traditional nanomaterials, are nontoxic materials because of their carbon-based nature (Ji et al. 2020). Furthermore, the surface of CDs is rich in functional groups of different nature that can function as binding sites for a variety of bioreceptors, which is peculiar for CDs employment as nanoprobe in biosensors, and finally, their simple and sustainable synthesis approaches are expected to achieve production on a large scale, which is crucial for CDs industrial development and commercial application in biosensing. Thus, from the above reported characteristics, CDs demonstrate great potential as components for biosensors (Campuzano et al. 2019; Haitang et al. 2014; Koutsogiannis et al. 2020; Fan et al. 2015; Qu et al. 2020).

It is well known that a biosensor is composed of three parts: a detector, for the recognition of the signal of the target analyte; a converter, for the conversion of the signal into an appropriate output; and a signal processor, for the analysis and the processing of the output signal (Perumal and Hashim 2014). Usually in the sensing systems reported in literature, CDs are employed as converters, i.e., they convert the signals of the target analyte into useful signals that could be recognized and processed by the signal processor. The abovementioned presence of various functional groups on CDs surface that can function as binding sites for specific

bioreceptors makes it possible to design biosensors, where CDs are directly sensitive to specific biomolecules (Zhou et al. 2020a; Wang et al. 2017) and function as both detectors and converters simultaneously without the further addition of bioreceptors.

In this context, the present chapter deals with CDs as new sustainable nanomaterials for biosensor applications and is organized into two main sections. The first one is devoted to describing the synthesis methods of CDs, mainly focusing on the green approaches and both the biocompatibility and toxicity of the obtained materials. The optical properties of CDs will also be treated by summarizing the most accepted explanations for their luminescence origin. The second section is devoted to the biosensing applications of CDs, focusing on the optical and electrochemical approaches.

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## 2 Synthesis and Optical Properties

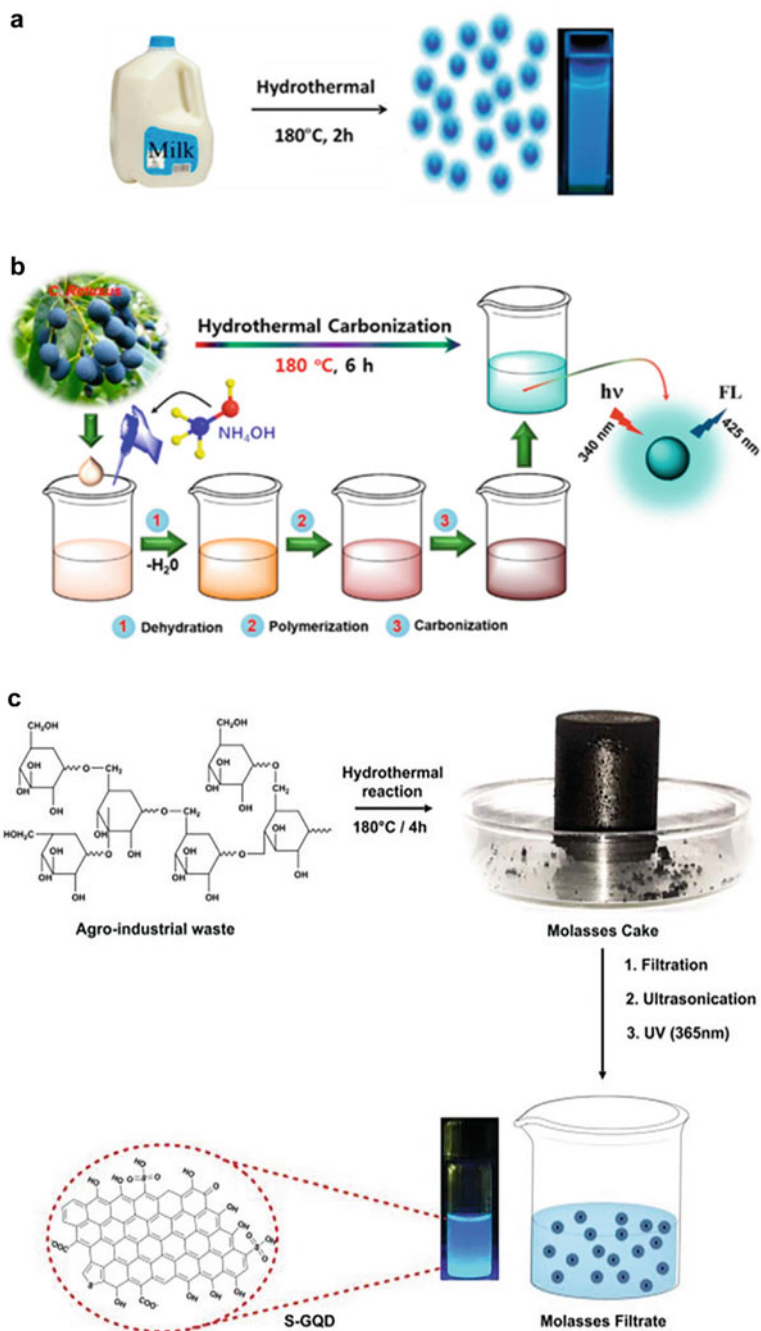
### 2.1 Synthesis

The sustainable strategies for the preparation of CDs are mainly devoted to the selection of “greener” precursors, i.e., materials obtained from biomass, and the development of new processes that should be both efficient and do not require high expenditures of external energy. In comparison with conventional synthesis methods for CDs, the approach based on biomass employs precursors that are natural and renewable in place of traditional carbon sources. On the one hand, the method presents disadvantages related to the variable properties and structures exhibited by the obtained CDs, which depend on the diversity of the employed renewable starting materials; on the other hand, the renewability of starting materials enables the inexpensive mass production of CDs, which is crucial for their industrial applications. In other words, the reaction temperature required in the biomass synthesis of CDs (usually 100–200 °C) is lower than that required for the preparation of CDs starting from traditional precursors (usually higher than 200 °C). Furthermore, the selection of solvents necessary for the CDs synthesis is related to the solubility of their precursor materials. Usually, materials obtained from biomass are soluble in aqueous media, while some traditional precursors usually require organic media because of their limited water solubility. The biomass synthesis of CDs is usually based on “bottom-up” strategies, currently including hydrothermal/solvothermal treatment and microwave irradiation. For example, Liu et al. (2017b) developed a green, facile, and low-cost approach based on the one-pot hydrothermal treatment of rose-heart radish for the preparation of fluorescent CDs with well-distributed size. The obtained CDs are highly fluorescent with a PL emission quantum yield of 13.6%, extremely biocompatible, chemically stable, and nontoxic. In another study, garlic was employed for the hydrothermal synthesis of nitrogen and sulfur co-doped CDs (Zhao et al. 2015). The obtained CDs are soluble in water, fluorescent in the blue region with a PL emission quantum yield of 17.5%, photo- and pH-stable, and noncytotoxic. Furthermore, they proved to be resistant to the interference of metal ions, biomolecules, and high ionic strength environments.

Wang and Zhou (2014) reported a green, simple, and sustainable strategy for the preparation of fluorescent nitrogen-doped CDs using milk as a precursor (Fig. 14.1a). In practice, by hydrothermal heating of milk, they produced mono-dispersed CDs of ca. 3 nm of diameter, which showed to be extremely fluorescent and did not present noticeable toxicity to the cells even at high concentrations of CDs (Wang and Zhou 2014). Another sustainable and green approach employed honey for the production of CDs with a quantum yield of about 19.8%, for imaging and sensing applications (Yang et al. 2014).

The prepared CDs are also nontoxic and proved to be both photo- and chemically stable. In another work by Edison et al. (2016), fluorescent nitrogen-doped CDs (N-CDs) were prepared through a fast and simple microwave approach. L-ascorbic acid and  $\beta$ -alanine were employed as carbon and nitrogen sources, respectively; the N-CDs exhibited excitation-dependent emission properties with a quantum yield of ca. 14% and low cytotoxicity. Eutrophic algal blooms were employed by Vadivel et al. (2016) as the carbon source for the development of a facile and rapid strategy for mass production of CDs. The produced CDs exhibit a QY of 13%, high water solubility, a nanosecond fluorescence lifetime characterized by high photostability and luminescence stability in different environments, low cytotoxicity, and excellent cell permeability. CDs were also prepared from the direct employment of fruit juice, like orange juice (Sahu et al. 2012), grape juice (Huang et al. 2014), banana juice (De and Karak 2013), *Chionanthus retusus* (*C. retusus*) fruit juice (Atchudan et al. 2017), etc. For example, Atchudan et al. (2017) developed a simple hydrothermal strategy for the preparation of N-CDs starting from *C. retusus* fruit juice (Fig. 14.1b). In another work, Atchudan et al. (2018) proposed a low-cost hydrothermal route for preparing N-CDs through the employment of *Phyllanthus acidus* (*P. acidus*) as a precursor. Furthermore, due to the growing attention towards environmental sustainability, some waste materials are employed for the synthesis of CDs. In fact, the concept of upcycling waste for the industrial production of significant amounts of CDs has recently gained a growing attraction. In particular, in order to reduce human impact on the environment through zero waste generation, agro-industrial waste, intended as products and byproducts, has become an object of interest. To this end, the work of Sangam et al. (2018) reports the development of a simple, economic, and large-scale synthetic strategy for the preparation of sulfur-doped GQDs (S-GQDs) employing as starting material biowastes of the second generation (Fig. 14.1c). The proposed approach, which could be considered a promising strategy for the sustainable preparation of CDs, employs sugarcane molasses as a green precursor. The obtained S-GQDs are highly crystalline, soluble in water, stable, highly fluorescent with a PL emission QY of 47%, and biocompatible. In another work, Zhang et al. (2015) propose a one-step hydrothermal method that employs egg white for the preparation of fluorescent CDs with a QY of 61%, which were used for the detection of  $\text{Fe}^{3+}$  ions and for living cell imaging.

**Toxicity** – Recent studies on CD toxicity report that, unlike heavy metal-based quantum dots, which are usually located in cells' cytoplasm, CDs enter into the nucleus. Furthermore, it was observed that the location of CDs in normal rat kidney (NRK) cells depends on their dose of exposure. In particular, upon exposure to



**Fig. 14.1** (a) Illustration of the formation process of carbon dots from milk by hydrothermal treatment. (Reproduced with permission from Wang and Zhou (2014), © 2014 American Chemical

3 mg mL<sup>-1</sup> CDs for 6 h, the CDs were located in the cytoplasm, while after exposure to 6 mg mL<sup>-1</sup> CDs for the same time, they could enter the nucleus (Song et al. 2019). Furthermore, it is interesting to note that after exposures for 6 h to CDs 3 and 6 mg mL<sup>-1</sup>, they did not induce the normal rat kidney cell apoptosis and necrosis, but they did cause autophagy and change the mitochondrial energy metabolism from aerobic to glycolytic metabolism (Song et al. 2019). It was also found that CDs cytotoxicity is mild; in fact, it was reported that the cell viability of HepG2 cells was greater than 90% after 4 h of incubation at a CDs concentration of 2 mg mL<sup>-1</sup>. Even more, it was observed that CDs helped HepG2 cells to clear hydroxyl radicals induced by H<sub>2</sub>O<sub>2</sub> (Wang et al. 2019).

## 2.2 Optical Properties

One of the most appealing features of CDs is their PL emission, which makes them good candidates as nanoprobes in optical biosensors. In general, optical biosensors convert the intangible information of the target analytes into recognizable optical signals, i.e., fluorescence intensity/wavelength and color change. As above reported, an optical biosensor requires that the wavelength of the nanoprobe should be in the near-infrared (NIR) region (Zhang et al. 2016) because the high energy of ultraviolet rays may damage living organisms (Sinha and Häder 2002; Moan and Peak 1989) and biological tissues easily absorb visible light. To this end, most studies report that through suitably designed synthetic approaches (Loi et al. 2017; Kozák et al. 2013), adequate selection of carbon sources (Ge et al. 2015), and doping with heteroatoms (Jiang et al. 2020a; Sun et al. 2019; Zhang et al. 2012), it is possible to modulate the PL emission of CDs from the visible to the NIR. For example, a series of CDs with different emissions was obtained by Bao et al. (2015) through the manipulation of the reaction conditions. Ding et al. (2016) developed a hydrothermal strategy followed by further separation via silica gel column chromatography to prepare CDs with tunable fluorescence, which goes from blue to red under single-wavelength UV light. Although the literature is rich in studies on the argument, the origin of the fluorescence of CDs is still not clear and continues to be the object of scientific discussion. In fact, CDs revealed themselves to be more complex systems than expected because they exhibit dissimilar optical properties that are related to the synthetic strategies, the employed precursors, and the post-synthesis treatments. According to recent reported studies, it is possible to consider three main points of view on the fluorescence origin of CDs: (i) surface-state emission, which

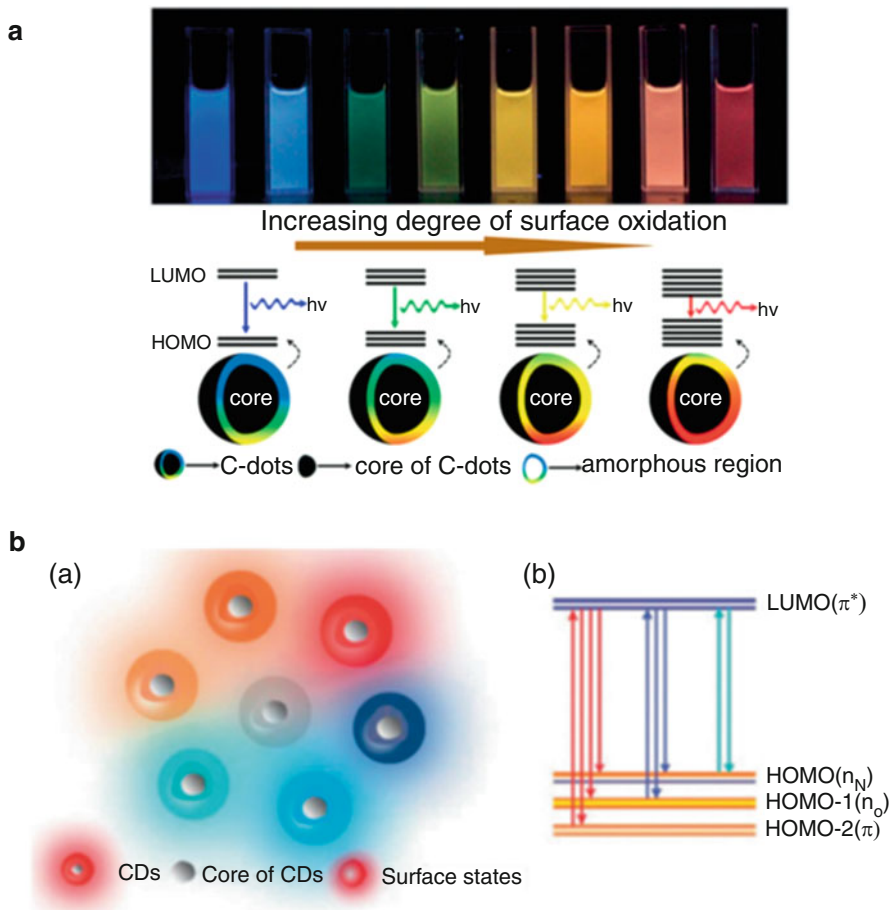


**Fig. 14.1** (continued) Society). (b) Schematic illustration for the preparation and formation mechanism of N-CDs from *Chionanthus retusus* (*C. retusus*) fruit juice. (Reproduced with permission from Atchudan et al. (2017), © 2016 Elsevier B.V.). (c) Schematic illustration of the mass synthesis of S-GQDs from agro-industrial waste. (Reproduced with permission from Sangam et al. (2018), © 2018 The Royal Society of Chemistry)

regards the carbon backbone and the surface functionalities (Song et al. 2015); (ii) core-state emission (quantum confinement), which depends on the crystallinity of CDs and their surface groups; (iii) molecular fluorescence, which is generated by fluorescent impurities/byproducts produced during CDs synthesis (Essner et al. 2018).

- (i) *Surface state emission* – It is the most widely accepted luminescence mechanism of CDs, and it is ascribed to their surface oxidation degree or their surface functional groups. Regarding CDs surface oxidation degree, Bao et al. (2011) showed that the oxygen content on CDs surface is responsible of the redshift of their PL emission. They suggested that a higher degree of surface oxidation may generate a greater number of surface defects that can act as exciton traps. Thus, the redshift of the PL emission is considered to be the result of the recombination of these trapped excitons. An example is the series of CDs prepared by Ding et al. (2016) through purification with column chromatography, which exhibit an excitation-independent fluorescence from blue to red (Fig. 14.2a). The authors showed that the surface oxidation degree of CDs gradually increases, going from blue to red emission. On the one hand, surface oxidation is considered responsible for the production of defects, which act as capture centers for excitons and cause a fluorescence emission that is related to the surface states (Bao et al. 2015). On the other hand, the incorporated oxygen species strongly affect the bandgap of CDs (Hu et al. 2015), i.e., by increasing the degree of surface oxidation of CDs, their bandgap reduces, causing the redshifted fluorescence emission (Fig. 14.2a). Green and yellow emissive CDs were prepared by Liu et al. (2017a) through a simple and sustainable room temperature method. These two CDs samples exhibit the same size distribution and chemical surface composition, but they are characterized by different surface oxidation degrees. In particular, the author observed that by increasing the surface oxidation degree, the emission wavelength shifted to lower energies, passing from 518 nm to 543 nm and ascribed the phenomenon to the decrease of their bandgaps. On the other hand, regarding the CDs surface functional groups, other research studies correlate the surface state emission of CDs with their surface functional groups, also known as molecular states, like C=O and C=N. It is suggested that different fluorophores or energy levels can be introduced in CDs by the functional groups present on their surface. The multicolor nitrogen-doped CDs (N-CDs) reported by Zhang et al. (2017) could be an example. The authors show that the PL emission can be modulated from dark blue to red or even white (Fig. 14.2b). As these full-color N-CDs exhibit similar oxygen content, the authors ascribed their PL emission to their surface functional groups rather than to their surface oxidation degree. In fact, they propose (Fig. 14.2b) that except for the HOMO-2( $\pi$ ) energy level, the functional groups (C=O and C=N) present on the surface of their N-CDs insert the two new energy levels HOMO-1 and HOMO, and determine the new level transitions from HOMO-1 and HOMO to LUMO ( $\pi^*$ ). A possible redshift of the emission is supposed to take place when the electrons in the N-related defect states return to the HOMO level.





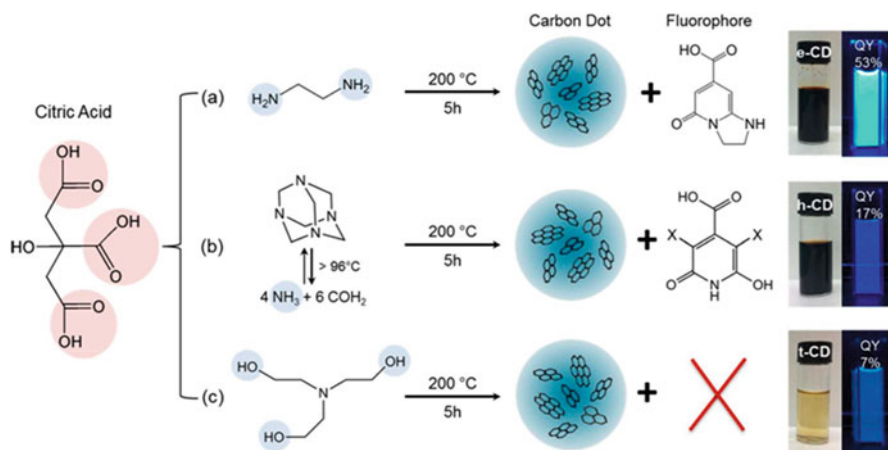
**Fig. 14.2** (a) Model for the tunable PL of CDs with different degrees of oxidation. (Reproduced with permission from Ding et al. (2016), © 2015 American Chemical Society). (b) (a) Structure of the multicolor CDs. (b) Schematic illustration of the proposed energy level and electron transition diagrams of the multicolor CDs. (Reproduced with permission from Zhang et al. (2017), © 2017 The Royal Society of Chemistry)

- (ii) *Core state emission (quantum confinement)* – The quantum confinement effect can be observed when the dimensions of a particle are comparable to the wavelength of the electron. Compared to the bulk material, in nanoparticles with diameters in the range of ca. 2–10 nm, the bandgap is increased because of the quantum size effect, and it causes different color emission depending on the small differences in the size of the particles. For example, three types of CDs, showing bright red, green, and blue PL emissions, respectively, under single ultraviolet-light excitation were reported by Jiang (2015a). These CDs exhibited similar chemical composition but different particle size distributions.

Thus, the authors ascribed the observed different PL emissions of these CDs to the quantum confinement effect. In another work, Li et al. (2010) observed that, when the oxygen on the surface of CDs is removed through hydrogen plasma, their PL emission remained unchanged. So, they suggested that the PL emission of their CDs may be ascribed to effects of quantum confinement and properties depending on particles dimensions. Sun et al. (2006) reported that the quantum confinement effect of the emissive energy traps present on CDs surface may be responsible for their PL emission upon surface passivation. In some reported cases, the PL emission of CDs is due to a combination of surface state and quantum confinement effect (Zhu et al. 2017). A series of CDs with different degree of surface oxidation and dimensions were synthesized by Bao et al. (2015). They proposed that the PL emission of their CDs is related to their surface oxidation degree and the  $\pi$ -electron system. In particular, a higher surface oxidation degree or an extended  $\pi$ -electron system corresponds to a smaller energy gap. Thus, they concluded that the redshift of the PL emission of their CDs is due to the increase in their particle size or their surface oxidation degree.

- (iii) *Molecular fluorescence* – Essner et al. (2018) observed that during the bottom-up synthesis strategies of preparation of CDs, some fluorescent impurities may form and contribute to increasing their PL emission (i.e., molecular fluorescence). In the CDs obtained employing citric acid and ethylenediamine, Song et al. (2015); Zhu et al. (2016) revealed the presence of the fluorescent molecule (imidazo[1,2-a]pyridine-7-carboxylic acid, 1,2,3,5-tetrahydro-5-oxo-, IPCA). They suggested that the observed high PL emission QY is ascribed to the contribution of IPCA and showed that their CDs consist of IPCA, polymers, and carbon cores. In further work, Schneider et al. (2017), through the preparation of three types of CDs employing citric acid and three different N-containing precursors, showed that the contribution to the PL emission of the obtained CDs samples may be ascribed to different molecular fluorophores. Furthermore, the authors showed that the molecular fluorophores are not reaction by-product free in the solution but are directly bonded to CDs and contribute to their emission behavior (Fig. 14.3).

Righetto et al. (2017) employed fluorescence correlation spectroscopy and time-resolved electron paramagnetic resonance spectroscopy to show that the PL emission of their CDs may be due to the small fluorophores present in solution or to their carbon-cores, depending on the excitation wavelength. In particular, in the excitation range from 320 to 450 nm the PL emission is mainly ascribed to the small fluorophores, while at excitation above 480 nm is dominated by poorly emitting carbon cores. Thus, they concluded that, although carbon cores are present, the PL emission of their CDs is mainly ascribed to the contribution of the small fluorophores present in the solution.



**Fig. 14.3** Synthesis conditions of citric acid-based CDs using three different nitrogen-containing precursors. (a) Reaction of citric acid and ethylenediamine, resulting in e-CDs and the fluorophore IPCA, as previously reported by Song et al. (2015); (b) Reaction of citric acid with hexamethylenetetramine, producing h-CDs and citrazinic acid and/or 3,5 derivatives (marked by -X), due to the decomposition of hexamethylenetetramine to ammonia and formaldehyde at temperatures exceeding 96 °C; (c) Reaction of citric acid and triethanolamine, resulting in t-CDs and no derivatives of citrazinic acid since the tertiary amine prohibits their formation. (a–c) Images of the purified reaction products under ambient light and corresponding diluted solutions under UV light excitation, which reveal blue emission with PL QYs as labeled on the graph. (Reproduced with permission from Schneider et al. (2017), © 2016 American Chemical Society)

### 3 Biosensing Applications

#### 3.1 Optical Biosensors

In general, an optical biosensor designed with CDs is based on the variation of the CDs optical properties (i.e., PL emission intensity or PL emission wavelength shift) caused by the interaction between the CDs and the target analytes whose related information can be provided depending on the impact (Ji et al. 2020). For the realization of an effective optical biosensor, the mechanism of sensing has to be taken into account for the application of the suited strategy. For CDs-based optical biosensors, there are two main strategies: (i) “on-off” strategy and (ii) “on-off-on” strategy.

- (i) *“on-off” strategy* – In an “on-off” strategy, the interaction between CDs and the target molecules causes a decrease of the intensity of their PL emission (PL quenching); this means that due to the quenching effect, the PL emission of CDs decreases with the increase of the concentration of the target analytes. Thus, the amount of the target analytes can be determined through a simple linear relationship, through calibration curves. The PL quenching mechanisms exploited in the design optical biosensors based on CDs are: Förster resonance

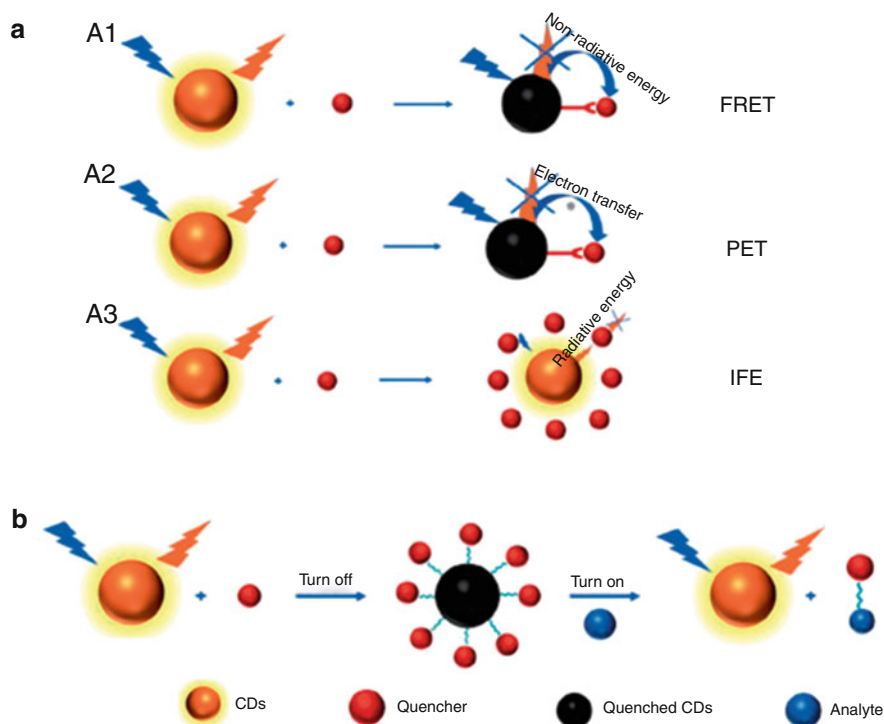
energy transfer (FRET), photoinduced electron transfer (PET), and inner filter effect (IFE), see Fig. 14.3. In a FRET quenching, the energy of the excited donor (i.e., CDs) is transferred without emission of radiation to the ground state of the proximal acceptor (i.e., quencher/target molecules). However, to have a FRET quenching, the following conditions have to be satisfied: (a) the energy of the excitation-state of the donor should be comparable with the one of the ground-state of the acceptor, i.e., the emission spectrum of the donor should overlap with the absorption spectrum of the acceptor; (b) the distance between the donor and the acceptor should be in the range from 1 nm to 10 nm; (c) the transition dipoles of the donor and acceptor molecules must be close to parallel; (d) the PL emission lifetime of the donor should be enough long to enable the energy transfer to occur.

In a PET quenching, an electron transfer from an electron donor (i.e., CDs) to an electron acceptor (i.e., the target analyte) occurs. Under the suitable conditions, the electrons photoexcited from CDs are transferred to the target analytes; thus, the PL emission of CDs is quenched because the number of the excited electron that return to the ground state is diminished. In an IFE process, the PL emission of CDs is blocked by the target analyte before reaching the detector of a fluorometer. In this case, the emission and absorption spectra of the donor (i.e., CDs) and acceptor (i.e., target analytes), respectively, need to overlap very well; thus, the target analytes can absorb the light emitted by the CDs, before reaching the detector, and cause a decrease in their PL intensity. The target analyte is determined through the analysis of the degree of PL quenching (Yang et al. 2019).

(ii) *“on-off-on” strategy* – In an “on-off-on” strategy, the CDs firstly interact with non-analytes that quench (turn-off) their PL emission which will be then restored (turn-on) by the addition of the analytes (Fig. 14.4). The analysis of the PL restoration degree of CDs gives the amount of the target analytes. The above reported PL quenching mechanisms (FRET, PET, or IFE) can be exploited for PL quenching of CDs by non-analyte quenchers. The interaction between the target analyte and the quencher has to be stronger than that between the quencher and CDs to cause the release of CDs from quenchers and thus restore the PL emission of CDs.

For example, Shen and Xia (2016) reported the realization of an optical biosensor for glucose, based on CDs obtained from phenylboronic acid. The interaction between glucose and the boronic functionalities present on CDs surface cause the quenching of their PL emission. The amounts of glucose levels in the body were determined through the degree of PL quenching, reaching a sensitivity that is 10–250 times higher than the one of the previous fluorescent nanosensing detection system based on boric acid.

In most cases, optical biosensors were developed for the detection of metal ions, because their interaction with CDs could effectively quench their PL via PET. A singular example is the sensitive detection of  $\text{Fe}^{3+}$  developed by Liu et al. (2016).

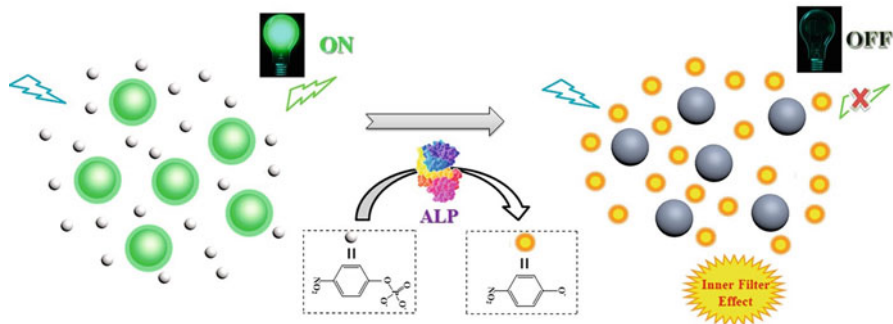


**Fig. 14.4** Typical optical biosensing strategies: (a) Three fluorescence-quenching mechanisms of “on-off” strategy, (A1) FRET, (A2) PET, and (A3) IFE. (b) Scheme for “on-off-on” strategy. (Reproduced with permission from Ji et al. (2020), © 2020 American Chemical Society)

They found that the PL quenching of CDs is not ascribed to a PET between  $\text{Fe}^{3+}$  and CDs but to the aggregation of CDs caused by the ferric ions. Other detection strategies developed by scientists are based on the detection of the products of the target analytes obtained by reactions with known mechanisms. It is possible to analyze the target analytes retrospectively from the information obtained from their products. For example, an efficient strategy for the detection of p-nitrophenylphosphate was developed by Li et al. (2016). The analysis was based on the IFE-quenching of the PL emission of CDs determined by p-nitrophenol, the catalytic product of p-nitrophenylphosphate (Fig. 14.5).

### 3.2 Electrochemical Biosensors

According to the International Union of Pure and Applied Chemistry (IUPAC) (Thevenot et al. 1999), an electrochemical biosensor is an integrated device that provides information on the target analytes by employing bioreceptors, which are put in direct contact with the electrochemical conduction element. In an

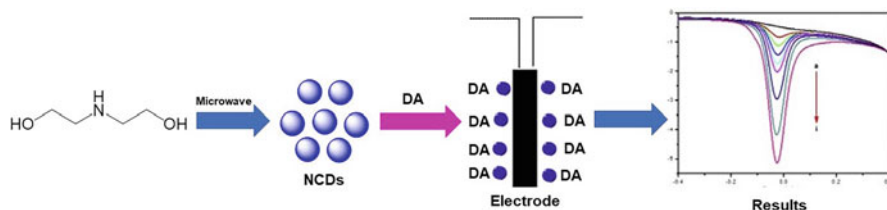


**Fig. 14.5** Working principle for alkaline phosphate (ALP) sensing based on inner filter effect (IFE). (Reproduced with permission from Li (2016), © 2016 American Chemical Society)

electrochemical biosensor, the biological information of the target analytes is directly converted into detectable electrical signals (i.e., current, potential, resistivity, capacitance, impedance, etc.). Another important aspect of electrochemical biosensors is that they can be easily miniaturized to satisfy the trend for modern medical applications, based on rapid diagnosis of diseases at low cost. Tool for electrochemical analyses with the dimensions of mobile phones able to realize simple testing without special training can be easily designed and fabricated, thanks to the advancements in the processes at microscale and nanoscale level. Furthermore, electrochemical biosensors are characterized by strong anti-interference capability and high sensitivity. As above reported, the surface of CDs is rich in functional groups of different nature that can function as binding sites for specific bioreceptors and that make CDs good candidates for the realization of electrochemical biosensors. Furthermore, CDs are good electronic conductors and are able to realize simple and rapid transfer of electrons between the sensing interface and the electrodes. In general, in an electrochemical biosensor, the electrode surface is directly modified with CDs, and the detection/analysis of the target analytes is performed through the collection of the signals (i.e., current) altered by the interaction between CDs and the target analytes. For example, N-CDs were prepared by Jiang et al. (2015b) through a sustainable strategy that did not employ organic solvent or catalyst. The CDs, whose surface was rich in carboxyl groups, were employed for the realization of an electrochemical biosensor for the direct detection of dopamine (Fig. 14.6). Thus, when an electrode modified with CDs is put in contact with dopamine, the interaction between CDs carboxylic groups and dopamine causes a change of the current of the electrode which shows a linear relationship with the concentration of dopamine.

The sensor was employed by the authors for the detection of dopamine in human fluids. The obtained linear range goes from  $5 \times 10^{-8}$  to  $8 \times 10^{-6}$  mol L<sup>-1</sup> and a detection limit is of  $1.2 \times 10^{-9}$  mol L<sup>-1</sup>. In a different study, a similar biosensor was developed by Jiang et al. (2015c) for the monitoring of dopamine in real time.

CDs can also be employed for the realization of photo-electrochemical biosensors where a simple photo-irradiation causes an electron transfer at the electrode (Wang



**Fig. 14.6** Scheme of an electrode loaded with CDs for the sensing of dopamine (DA). (Reproduced with permission from Jiang (2015a), © 2015 Elsevier Inc.)

et al. 2018b). For example, the first self-powdered photo-electrochemical biosensor based on N-CDs/TiO<sub>2</sub> for detection of chlorpyrifos was developed by Cheng et al. (2019). They found that the photocurrent response under visible light of the CDs-based electrode is about 42 times higher than the one without the CDs.

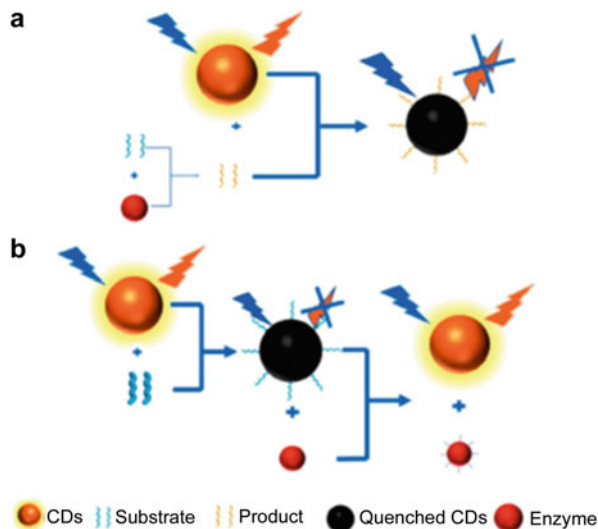
### 3.3 Enzymatic Biosensors

Biosensors based on the combination of CDs and enzymes were also designed and developed, by exploiting the simplicity and specificity of the enzymatic catalytic reactions. As above reported, indirect detection of the target analytes could be realized by the analysis of the variations of the signal caused by the interaction between the products (or side products) of the enzymatic reaction and CDs. Furthermore, enzymatic biosensors based on CDs have high sensing specificity, as the reaction catalyzed by enzymes is very specific. Thus, they could be very useful for the detection of particular biomolecules and enzymes.

*Enzymatic optical biosensors* are based on the PL quenching (FRET, PET, or IFE mechanisms) caused by the interaction between CDs and the product of the enzymatic reaction of the target analytes (Fig. 14.7).

For example, H<sub>2</sub>O<sub>2</sub> is the product of the enzymatic oxidation reactions of acetylcholine, uric acid, and glucose; thus, the amounts of these target analytes in human fluids could be determined through the analysis of the PL quenching due to the interaction between CDs and H<sub>2</sub>O<sub>2</sub> (Cho and Park 2019; Wang et al. 2016; Ren et al. 2015; Wang et al. 2018b). An interesting CD-based enzymatic biosensor was designed and developed by Lu et al. (2016) for the determination of the  $\beta$ -glucuronidase. They exploited the IFE quenching effect caused by the interaction between CDs and p-nitrophenol, which is the product of the enzymatic reaction of 4-nitrophenyl- $\beta$ -D-glucuronide. On the other side, the enzymatic biosensors could be employed for the detection and evaluation of the catalytic performance of specific enzymes. In this case, the “on-off-on” strategy was exploited for the design/realization of the biosensors (Fig. 14.7). The strategy simply consists of the formation of a complex between the substrate and the CDs, which causes the PL quenching of CDs; then the substrate is decomposed upon the interaction with the specific enzyme and the PL emission of CDs is restored. Thus, the presence and the amount of the specific

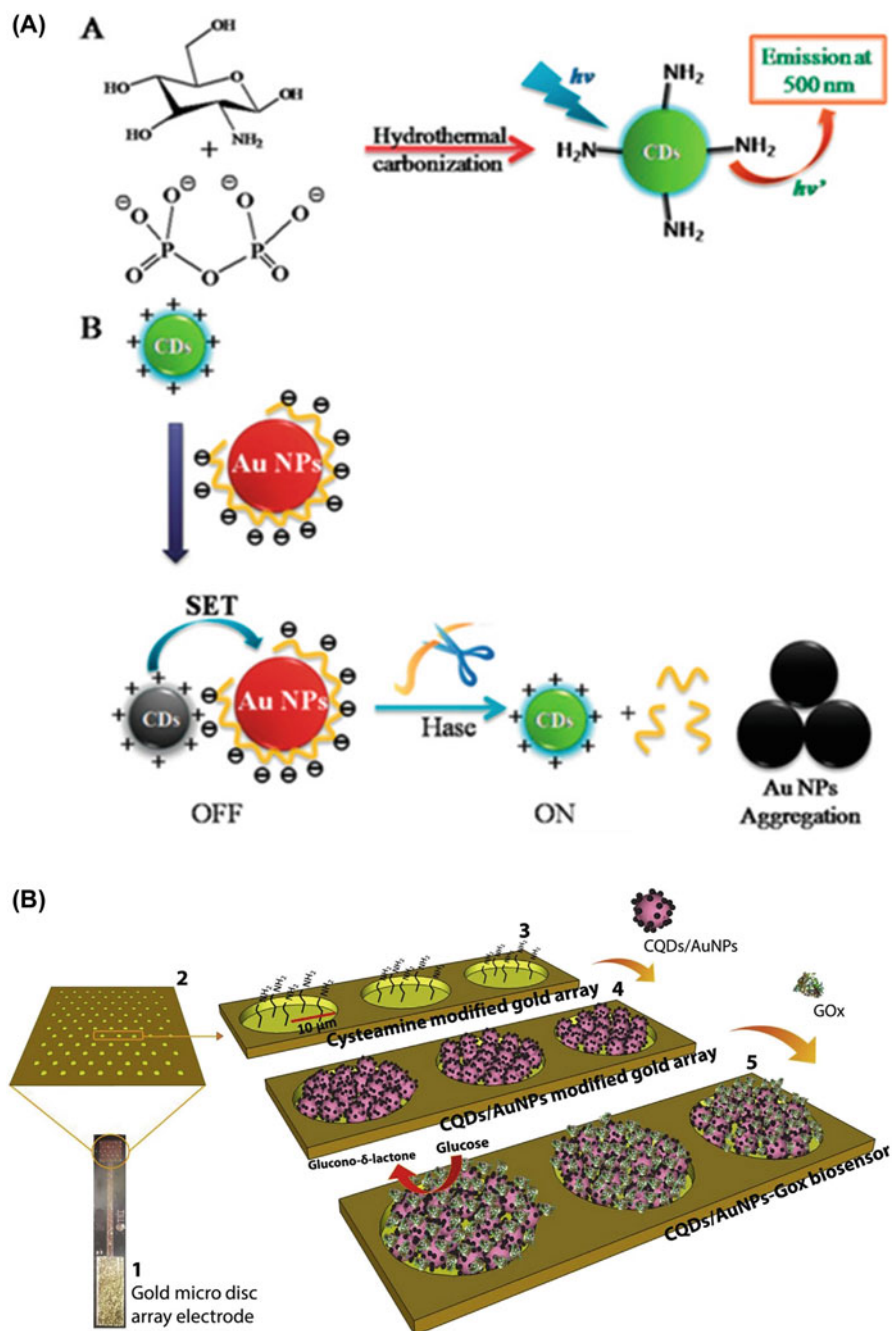
**Fig. 14.7** Two sensing strategies of CD biosensors based on enzymatic reactions. (Reproduced with permission from Ji et al. (2020), © 2020 American Chemical Society)



enzyme could be determined by the analysis of the restored PL emission of CDs. An example is a biosensor for the detection of hyaluronidase reported by Liu et al. (2015). It is based on CDs, whose surface consists of positively charged functional groups and gold nanoparticles (AuNPs, known to be negatively charged) functionalized with hyaluronic acid. The PL emission of the CDs is firstly quenched by the interaction with AuNPs and then restored by the introduction of hyaluronidase that determined the decomposition of hyaluronic acid (Fig. 14.8a).

*Enzymatic electrochemical biosensors* are based on the determination of the target analysts through the analysis of the variations of the electrochemical signal caused by the enzymatic reactions. On the one hand, the employment of CDs in these systems provides rich binding sites for enzymes that favor their stable absorption on the electrode, and on the other hand, it improves the transmission of the electrical signals from the interfaces to the electrode because CDs are also good electron transfer materials. For example, the redox reaction between oxidoreductase and its substrates could be exploited for the design of biosensors based on the electrochemical detection of its substrates, like glucose, galactose, and  $H_2O_2$ . When an electrode modified with an oxidase is put into contact with a solution containing the substrate, a redox reaction takes place at the electrode interface that causes the transfer of an electron. Thus, through the analysis of the electrode current variations determined by this electron transfer, it is possible to detect the target analytes (i.e., oxidoreductase substrates). Following this strategy, various CDs-based electrochemical biosensors were designed and realized for the detection of oxidoreductase substrates (i.e., glucose (Buk and Pemble 2019) (Fig. 14.8b),  $H_2O_2$  (Wang et al. 2015), and galactose (Sharma et al. 2019)).





**Fig. 14.8** (a) The schematic illustration of the synthetic process of amino-functionalized CDs (A) and the use of prepared CDs and Au NPs as a SET biosensor system for hyaluronidase

## 4 Conclusions

In summary, with this chapter, it was possible to show how the peculiar properties of CDs make them extremely versatile nanomaterials for application in biosensing. In fact, they exhibit an excellent intrinsic fluorescence, which could be modulated from the visible to NIR, and their surface is rich in functional groups, which enable their functionalization with different bioreceptors and functionalities (i.e., enzymes, etc.); they are also characterized by extremely low toxicity and excellent biocompatibility useful for their real-world biological applications. On the other hand, CDs are also expected to reduce the cost of biosensors by replacing both the noble metal substrates and the well-known quantum dots based on metal chalcogenides. In fact, their synthesis is based on simple and sustainable approaches that could employ cheap and “greener” starting materials coming from biomass or agro-industrial waste. They proved to be applied with excellent results both in optical and electrochemical biosensors. As a final remark, the gap between the development of CD-based optical biosensors and other types of CD-based biosensors, like electrochemical ones, could be highlighted. Compared with optical biosensors, the development of electrochemical ones should be improved, also in light of their excellent detection limits, strong anti-interference capability, and miniaturization possibilities.

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**Fig. 14.8** (continued) (B) (Reproduced with permission from Liu (2015), © 2015 The Royal Society of Chemistry); (b) Schematic diagram of the biosensor preparation process: (1) the bare individual disk array electrode, (2) magnified surface of the array, (3) amine-functionalized gold surface after cysteamine modification, (4) CQDs/AuNPs attached surface, and (5) GOx enzyme immobilized overall CQDs/AuNPs-GOx biosensor. Note that the size of the electrodes, nanomaterials, and biomolecules showed are not drawn to scale. (Reproduced with permission from Buk (2019), © 2018 Elsevier Ltd.)

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